JC19 Rec'd PCT/PTO 24 MAY 2001

FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	
0032-0261P	
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	
09/856662 NEW	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP99/05527	October 7, 1999	November 26, 2001

TITLE OF INVENTION

METHOD FOR TYPING OF HLA CLASS I ALLELES

APPLICANT(S) FOR DO/EO/US

MORIBE, Toyoki and KANESHIGE, Toshihiko

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39 (1).
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau. WO 00/31295
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is transmitted herewith.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☒ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 20. below concern document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98./-1449 and International Search Report (PCT/ISA/210)
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:
    Sequence Listing
    Six (6) sheets of formal drawings

JC03 Rec'd PCT/PTO 24 MAY 2001

U.S. APPLICATION NO (if known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
09/856662 NEW	PCT/JP99/05527	0032-0261P

21. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. **$1,000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO. **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4). **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 0	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	34 - 20 =	14	X **$18.00**	$ 252.00	
Independent Claims	4 - 3 =	1	X **$80.00**	$ 80.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)	Yes		+ **$270.00**	$ 270.00	
TOTAL OF ABOVE CALCULATIONS =				$ 1,462.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$ 0	
SUBTOTAL =				$ 1,462.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐20 ☐30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0	
TOTAL NATIONAL FEE =				$ 1,462.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 1,502.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ **1,502.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account. No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-2448.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

Send all correspondence to:
Birch, Stewart, Kolasch & Birch, LLP or Customer No. 2292
P.O. Box 747
Falls Church, VA 22040-0747
(703)205-8000

Date: May 24, 2001

By _____
Raymond C. Stewart, #21,066

/rem

PATENT
0032-0261P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	MORIBE, Toyoki et al.	Conf.:	
Int'l. Appl. No.:	PCT/JP99/05527		
Appl. No.:	NEW	Group:	
Filed:	May 24, 2001	Examiner:	
For:	METHOD FOR TYPING OF HLA CLASS I ALLELES		

PRELIMINARY AMENDMENT

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, DC 20231

May 24, 2001

Sir:

The following Preliminary Amendments and Remarks are respectfully submitted in connection with the above-identified application.

AMENDMENTS

IN THE SPECIFICATION:

Please amend the specification as follows:

Before line 1, insert --This application is the national phase under 35 U.S.C. § 371 of PCT International Application No. PCT/JP99/05527 which has an International filing date of October 7, 1999, which designated the United States of America and was published in English.

IN THE CLAIMS:

Please amend the claims as follows:

7. (Amended) The method for typing of the HLA class I alleles claimed in claim 5, wherein the temperature for washing after hybridization of the amplified products by the PCR method with the immobilized DNA probes and/or after the binding reaction of the label of the amplified products with the enzyme-conjugate is room temperature.

8. (Amended) The method for typing of the HLA class I alleles claimed in claim 1, wherein the amino-modified DNA probe which can specifically hybridize with at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, is selected from the group consisting of A98T (SEQ ID No.:1), A98A (SEQ ID No.:2), A160A (SEQ ID No.:3), A239A (SEQ ID No.:4),A238A (SEQ ID No.:5), A240T (SEQ ID No.:6), A257TC (SEQ ID No.:7), A259AC (SEQ ID No.:8), A270T (SEQ ID No.:9), A282C (SEQ ID No.:10), A290T (SEQ ID No.:11), A299T (SEQ ID No.:12), A302G (SEQ ID No.:13), A355G (SEQ ID No.:14), A362TA (SEQ ID No.:15), A362TT (SEQ ID No.:16), A368A (SEQ ID No.: 17), A368G (SEQ ID No.: 18), A368T (SEQ ID No.: 19), A402G (SEQ ID No.:20), A423T (SEQ ID No.:21), A448C (SEQ ID No.: 22), A485A (SEQ ID No.:23), A524G (SEQ ID No.:24), A526T (SEQ ID No.:25), A527A (SEQ ID No.:26), A538CG (SEQ ID No.:27), A539A (SEQ ID No.:28), A539T (SEQ ID No.:29), A555T (SEQ ID No.:30), A559G (SEQ

ID No.:31), A570CG (SEQ ID No.:32), A570GT (SEQ ID No.:33), A779A (SEQ ID No.:34), A843A (SEQ ID No.:35), BL1 (SEQ ID No.:36), BL3 (SEQ ID No.:37), BL4 (SEQ ID No.:38), BL5 (SEQ ID No.:39), BL9 (SEQ ID No.:40), BL10 (SEQ ID No.:41), BL11 (SEQ ID No.:42), BL24 (SEQ ID No.:43), BL25 (SEQ ID No.:44), BL34 (SEQ ID No.:45), BL35 (SEQ ID No.:46), BL36 (SEQ ID No.:47), BL37 (SEQ ID No.:48), BL38 (SEQ ID No.:49), BL39 (SEQ ID No.: 50), BL40 (SEQ ID No.:51), BL41 (SEQ ID No.:52), Bl42 SEQ ID No.:53), BL56 (SEQ ID No.:54), BL57 (SEQ ID No.:55), BL78 (SEQ ID No.:56), BL79 (SEQ ID No.:57), BL222A (SEQ ID No.: 58), BL272GA (SEQ ID No.:59), BL226G (SEQ ID No.:60), BL292G (SEQ ID No.:61), BL292T (SEQ ID No.:62), BL361G (SEQ ID No.:63), BL409T (SEQ ID No.:64), BL512T (SEQ ID No.:65, BL538CG (SEQ ID No.:66), BL538G (SEQ ID No.:67), CC (SEQ ID No.:68), A-12 (SEQ ID No.:69), A-2 (SEQ ID No.:70), A-3 (SEQ ID No.:71), A-4 (SEQ ID No.:72), A-54 (SEQ ID No.:73), B-1 (SEQ ID No.:74), B-2 (SEQ ID No.:75), C-12 (SEQ ID No.:76), C-24 (SEQ ID No.:77), C-33 (SEQ ID No.:78), C-43 (SEQ ID No.:79), 134-g (SEQ ID No.:80), 134-A2 (SEQ ID No.:81), 353TCA1 (SEQ ID No.:82), 343A (SEQ ID No.:83), A34 (SEQ ID No.:100), A282CT (SEQ ID No.:101), A290TR (SEQ ID No.:102), A302GR (SEQ ID No.:103), A414A (SEQ ID No.:104), A468T (SEQ ID No.:105), A489A (SEQ ID No.:106), A502C (SEQ ID No.:107), A538TG (SEQ ID No.:108), BL39R (SEQ ID No.:109) Bl50 (SEQ ID No.:110), BL77 (SEQ ID No.:111), BL272A (SEQ ID No.:112), BL263T (SEQ ID No.:113), BL527A (SEQ ID No.:114), BL570GT (SEQ ID No.:115), RA-2 (SEQ ID No.:116), RA-41 (SEQ ID No.:117), RB-28 (SEQ ID No.:118), 201g1 (SEQ ID No.:119), C206gR

(SEQ ID No.:120), R341A (SEQ ID No.:121), R343g3 (SEQ ID No.:122), 353TCC (SEQ ID No.:123), 361T1 (SEQ ID No.:124), 361T368g (SEQ ID No.:125), 361T368T1 (SEQ ID No.:126), 369C (SEQ ID No.:127), 387g1 (SEQ ID No.:128), 526AC2 (SEQ IDNo.:129), 538gAC (SEQ ID No.:130), complementary strands thereof and nucleic acids which comprises one to several bases are deleted from or added to the end of them.

9. (Amended) The method for typing of the HLA class I alleles claimed in claim 1, which comprises primers capable of amplifying all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles, or primers specific to the common sequence to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles, is selected from A2-5T (SEQ ID No.:84), A3-273T (SEQ ID No.:85), A4-8C (SEQ ID No.:86), A4-254G (SEQ ID No.:87), BASF-1 (SEQ ID No.:88), BASR-1 (SEQ ID No.:89), CGA011 (SEQ ID No.:90), CGA012 (SEQ ID No.:91), Ain3-66C (SEQ ID No.:92), 5BCIn37-34C (SEQ ID No.:96), 5BCIn37-24g (SEQ ID No.:97), and 5BCIn37-34g2 (SEQ ID No.:99).

12. (Amended) A kit for typing of the HLA class I alleles, which is used for the method claimed in claim 1.

13. (Amended) A reagent for typing of the HLA class I alleles, which is used for the method claimed in claim 1.

21. (Amended) (Added)The method claimed in claim 18, wherein the probes are hybridized with amplified products by the PCR method.

23. (Amended) (Added) The method claimed in claim 18, wherein nucleic acids are hybridized with the probes immobilized on a support.

24. (Amended) (Added) The method claimed in claim 21, which comprises hybridizing the amplified products obtained by the PCR method with the immobilized DNA probes, adding an enzyme-conjugate which specifically bonds to a label of the amplified products thereto at the same time or after the hybridization, and adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate to the mixture, to detect as signals whether or not the amplified products are hybridized with the immobilized DNA probes.

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application. The claims have also been amended to delete improper multiple dependencies and to place the application into better form for examination. Entry of the present amendment and favorable action on the above-identified application are earnestly solicited.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By 
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RCS/rem
0032-0261P

(Rev. 02/12/01)

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The Specification has been amended to provide cross-referencing to the International Application.

The claims have been amended as follows:

7. (Amended) The method for typing of the HLA class I alleles claimed in[clims 5 or 6] claim 5, wherein the temperature for washing after hybridization of the amplified products by the PCR method with the immobilized DNA probes and/or after the binding reaction of the label of the amplified products with the enzyme-conjugate is room temperature.

8. (Amended) The method for typing of the HLA class I alleles claimed in[any one of claims 1 to 7] claim 1, wherein the amino-modified DNA probe which can specifically hybridize with at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, is selected from the group consisting of A98T (SEQ ID No.:1), A98A (SEQ ID No.:2), A160A (SEQ ID No.:3), A239A (SEQ ID No.:4),A238A (SEQ ID No.:5), A240T (SEQ ID No.:6), A257TC (SEQ ID No.:7), A259AC (SEQ ID No.:8), A270T (SEQ ID No.:9), A282C (SEQ ID No.:10), A290T (SEQ ID No.:11), A299T (SEQ ID No.:12), A302G (SEQ ID No.:13), A355G (SEQ ID No.:14), A362TA (SEQ ID No.:15), A362TT (SEQ ID No.:16), A368A (SEQ ID No.: 17), A368G (SEQ ID No.: 18), A368T (SEQ ID No.: 19), A402G (SEQ ID No.:20),

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No.:105), A489A (SEQ ID No.:106), A502C (SEQ ID No.:107), A538TG (SEQ ID No.:108), BL39R (SEQ ID No.:109) Bl50 (SEQ ID No.:110), BL77 (SEQ ID No.:111), BL272A (SEQ ID No.:112), BL263T (SEQ ID No.:113), BL527A (SEQ ID No.:114), BL570GT (SEQ ID No.:115), RA-2 (SEQ ID No.:116), RA-41 (SEQ ID No.:117), RB-28 (SEQ ID No.:118), 201g1 (SEQ ID No.:119), C206gR(SEQ ID No.:120), R341A (SEQ ID No.:121), R343g3 (SEQ ID No.:122), 353TCC (SEQ ID No.:123), 361T1 (SEQ ID No.:124), 361T368g (SEQ ID No.:125), 361T368T1 (SEQ ID No.:126), 369C (SEQ ID No.:127), 387g1 (SEQ ID No.:128), 526AC2 (SEQ IDNo.:129), 538gAC (SEQ ID No.:130), complementary strands thereof and nucleic acids which comprises one to several bases are deleted from or added to the end of them.

9. (Amended) The method for typing of the HLA class I alleles claimed in[any one of claims 1 to 8] claim 1, which comprises primers capable of amplifying all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles, or primers specific to the common sequence to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles, is selected from A2-5T (SEQ ID No.:84), A3-273T (SEQ ID No.:85), A4-8C (SEQ ID No.:86), A4-254G (SEQ ID No.:87), BASF-1 (SEQ ID No.:88), BASR-1 (SEQ ID No.:89), CGA011 (SEQ ID No.:90), CGA012 (SEQ ID No.:91), Ain3-66C (SEQ ID No.:92), 5BCIn37-34C (SEQ ID No.:96), 5BCIn37-24g (SEQ ID No.:97), and 5BCIn37-34g2 (SEQ ID No.:99).

12. (Amended) A kit for typing of the HLA class I alleles, which is used for the method claimed in[any one of claims 1 to 9] claim 1.

13. (Amended) A reagent for typing of the HLA class I alleles, which is used for the method claimed in[any one of claims 1 to 9] claim 1.

21. (Amended) (Added)The method claimed in[any one of claims 18 to 20] claim 18, wherein the probes are hybridized with amplified products by the PCR method.

23. (Amended) (Added) The method claimed in[any one of claims 18 to 22) claim 18, wherein nucleic acids are hybridized with the probes immobilized on a support.

24. (Amended) (Added) The method claimed in[any one of claims 21 to 23] claim 21, which comprises hybridizing the amplified products obtained by the PCR method with the immobilized DNA probes, adding an enzyme-conjugate which specifically bonds to a label of the amplified products thereto at the same time or after the hybridization, and adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate to the mixture, to

detect as signals whether or not the amplified products are hybridized with the immobilized DNA probes.

DESCRIPTION

METHOD FOR TYPING OF HLA CLASS I ALLELES

Technical Field

HLA (Human Leukocyte Antigen) that is Human major histocompatibility antigen, is expressed on membranes of imuunocompetent cells, presents processed peptides derived from exogenous and endogenous antigens to T lymphocytes, and functions as a marker to recognize self and non-self. The present invention relates to a method, a reagent and a kit for typing of the HLA class I alleles. This invention is especially useful for judgement of compatibility between a donor and a recipient in organ transplantation, and for association analysis between the HLA class I genes and various types of diseases in the clinical and medical field. This invention enables us to easily automate and mechanize detection and determination of the HLA class I alleles.

Background of Art

Typing of the HLA antigens has been mainly performed by the serological method using human alloantibodies. By using the specific antibodies to each HLA antigen which are contained in cord blood or serum from subjects who have frequently undergone blood transfusion, complement-mediated cytotoxicity is caused in the antigen-antibody reaction. It changes permeability of positive cell membranes to take an eosinic pigment into the cell, resulting in being detected as colored and expanding cells with a microscope. It is possible to type HLA-A, HLA-B and HLA-C antigens belonging to HLA class I, and HLA-DR and HLA-DQ antigens belonging to HLA class II by this method.

However, this method has problems in terms of collection, quality control and supply of the specific antibodies. Furthermore, the survival rate of cells is utilized as an indicator for judgement in this method. Therefore, poor conditions of subjects, for example, a low survival rate of cells caused by disease or influence by passage of time after blood collection, lead to decrease of credibility for results of testing.

In recent years, a development of molecular biotechnology has enabled us to analyze the region of genes encoding the HLA antigens. That has clarified the correspondence between the HLA antigens and the sequences of the HLA genes. This means it has been possible to identify the HLA antigen type by analyzing the specific sequences of the HLA genes (DNA typing). Especially, PCR (polymerase chain reaction) method which can high-sensitively detect a slight change of sequences is utilized to type the HLA-DR, -DQ, or -DP genes belonging to HLA class II. Several PCR-based typing methods for HLA class II DNA such as PCR-SSOP (Sequence-Specific Oligonucleotide Probe) method, PCR-RFLP (Restriction Fragment Length Polymorphism) method, PCR-SSP (Sequence-Specific Primers) method and PCR-SSCP (Single Strand Conformation Polymorphism) method have been developed. In all these methods, the gene region to analyze is amplified by the PCR method and then the variable region in the sequences of the amplified products is analyzed by combination with another methods in order to distinguish the genotype. The HLA class II DNA typing method makes it possible to classify the HLA type at the allele level in addition to classification by the classical serological method using human alloantisera.

Development of the PCR based-method for HLA class I DNA typing is delayed remarkably, comparing with HLA class II typing. The reasons are as follows: (1) While almost all the class II gene mutations (gene substitutions),

including those which reflect the specificity of antigens, concentrate in the region of the exon 2, the class I gene mutations are interspersed among the regions of the exons 2 and 3, or the exon 4. (2) The HLA class I genes, including non-classical genes (HLA-E, -F and -G) and pseudogenes (HLA-H, -J, -K and -L), are highly homologous among them.

To date, several HLA class I DNA typing methods have been reported. However, all these methods require complicated manipulation, strict reaction condition and skill. Those are not suitable for handling a large number of samples and offer only low resolution HLA typing. Furthermore, the typing methods for each gene are not standardized.

Disclosure of Invention

The purpose of this invention is to solve problems of the manipulation of HLA class I locus antigen typing by the classical serological method, and to prodive a method, a kit and a reagent for classifing the subtype of the HLA class I antigens at the allele level (allele typing), which has not been distinguished by the classical method. Furthermore, the aim of this invention is to provide a method for typing of the HLA class I alleles which can automate and machanize easily.

As a result of intensive studies for these subjects, the inventors have established primers which can amplify all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles and specific primers to the common sequences among all alleles in the group consisting of the specific HLA-A alleles or the specific HLA-B alleles. The inventors have established probes which can specifically hybridize with the sequence of at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele. The inventors have found out that it is possible to distinguish the HLA class

I antigen or allele, by hybridizing the PCR amplified products derived from the specific HLA class I allele or the specific group with the DNA probes described above which are immobilized on wells of microtiter plates, adding an enzyme-conjugate which can specifically bond to a label of the amplified products at the same time as or after the hybridization, and adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate to the mixture, to detect as signals whether or not the amplified products are hybridized with the immobilized DNA probes. Thus, they have accomplished this invention.

The main embodiment of this invention is a method for typing of HLA class I alleles, which comprises the following steps from (a) to (d).

(a) A step, using HLA class I gene or nucleic acids containing their fragment for a template,

(1) To non-selectively amplify all HLA-A alleles, all HLA-B alleles or all HLA-C alleles by a PCR method using a primer pair which can amplify all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles, or

(2) To selectively amplify a specific group consisting of specific HLA-A alleles or specific HLA-B alleles by a PCR method using a primer pair which is specific to the common sequence to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles,

(b) A step to add the above products amplified by the PCR method to wells of microtiter plates, wherein each well is modified with a carboxyl group to covalently immobilize amino-modified DNA probes which can specifically hybridize with the sequence of at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, and to

hybridize the amplified products with the immobilized DNA probes, wherein the DNA probes are selected depending on the above amplified specific HLA class I gene or group;

(c) A step to detect as signals whether or not the amplified products are hybridized with the immobilized probes; and

(d) A step to determine the type of the HLA class I allele based on the signal pattern detected at the step (c) according to the Typing Table.

The PCR amplification of the target gene at the step (a), can be classified into 2 steps. One is a step to non-selectively amplify all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles by the PCR method using a primer pair which can amplify all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles. The other is a step to selectively amplify the specific group consisting of the specific HLA-A allele group or the specific HLA-B allele group by the PCR method using a primer pair which is specific to the common sequences to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles. At the former step, PCR primers are designed to be specific to the common sequences, which are within the region of all alleles belonging to the HLA-A allele, the HLA-B allele or the HLA-C allele, or ahead and behind the region. At the latter step, PCR primers are designed to be specific to the common sequences to all alleles included in the specific group in order to amplify the specific group. When the specific group is selectively amplified in the presence of some groups, the primers described above don't need to be used for both a sense primer and an antisense primer of a primer pair corresponding to the specific group. It is possible to use the specific primer to the specific group for one of primers and the specific primer to all the groups for the other. The

latter step can be performed according to the reference described by the inventors (Tissue Antigens 1997, Vol.50, 535-545). A method to selectively amplify alleles encoding the HLA-A2 antigen or the HLA-B40 antigen as a group is disclosed in the present description.

At the step (a), the PCR-amplified products derived from the allele belonging to the HLA-A alleles, the HLA-B alleles or the HLA-C alleles, or from the specific group, are produced. But it is not possible to distinguish the type of the HLA class I allele at the step. The hybridization reaction at the step (b) using the specific DNA probes is applied to the following steps.

The Typing Table at the step (d) is made using signal patterns obtained by hybridizing the PCR amplified products from samples whose HLA class I antigen types or allele types are known, with DNA probes which can specifically hybridize with the sequence of at least one specific HLA class I allele. Persons skilled in the art can make easily the Typing Table. As the Typing Table, Figures 1 to 6 can be referred. If someone wants to use DNA probes, which are not described in this description, another Typing Table can be used. The Typing Table is made from signal patterns obtained by hybridizing the PCR amplified products from samples whose HLA class I antigen types or allele types are known, with another DNA probe. As described above, persons skilled in the art can also make easily these Typing Tables. It should be considered that each sample has the HLA class I allele type in a homozygous or heterozygous state, when the HLA class I allele type is distinguished according to the Typing Tables.

In a perferable embodiment, the PCR method at the step (a) is performed by using a primer pair in which at least one of them is labeled, in order to detect whether or not the amplified products hybridize with immobilized

DNA probes as signals at the step (c) described above. In the other embodiment, the above PCR can be performed by using 4 kinds of deoxyribonucleotide triphosphate (dNTP) in which at least one of them is labeled. As a substance used for labeling, a radioisotopic substance, or a non-radioisotopic substance such as a biotin or a digoxigenin, can be utilized.

In a preferable embodiment, at the step (b) or (c) described above, the hybridization of the products amplified by the PCR method with the immobilized DNA probe is performed by addiing an enzyme-conjugate which can specifically bond to a label of the amplified products is added at the same time as hybridization or after, and the amplified products hybridizing with the immobilized DNA probe is detected as signals by adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate which can specifically react with the enzyme. When a peroxidase-conjugated streptavidin is used as an enzyme-conjugate, the signal can be immediately detected after washing by adding an enzyme-conjugate at the same time as hybridization.

In a preferable embodiment, at least one of a primer pair at the step (a) described above is biotinylated, and an enzyme-conjugate which can specifically bond to the biotinylated label at the step (b) or (c) is an enzyme-conjugated streptavidin, for example, a peroxydase-conjugated streptavidin or an alkaline phosphatase-conjugated streptavidin.

In a preferable embodiment, the hybridization of the products amplified by the PCR method with immobilized DNA probes is performed in a solution containing formamide at the step (b) described above. The formamide concentration of the solution described above (hybridization buffer) is from 5% to 30%, and from 10% to 25% as a preferable concentration. The concentration can be changed according to the sequence, the length and the

type of the used DNA probe. The most preferable formamide concentration is about 20%.

In a preferrable embodiment, the hybridization at the step (b) is performed in a solution containing formamide at the temperature of the 37℃. The preferable temperature is from 32℃ to 42℃. The temperature can be changed according to the sequence, the length and the type of the used DNA probe as mentioned above for the formamide concentration. The most desirable temperature is about 37℃. Hybridization is usually performed at comparatively high temperature, at about 65℃, to improve the specificity. By using the solution containing formamide, the reaction can be performed at low temperature, at about 37℃.

In a preferable embodiment, when the solution containing formamide is used for the hybridization at the step (b) described above, the temperature for washing after hybridization of the amplified products by the PCR method with immobilized DNA probes and/or after binding a label of the amplified products with an enzyme-conjugate is performed at room temperature. Namely, washing can be performed at low temperaure like room temperature as by using the solution containing formamide, as well as the above hybridization.

The amino-modified DNA which can specifically hybridize with at least one specific HLA-A allele, used at the step (b) in this invention, can be selected from the group consisting of A98T (SEQ ID No.:1), A98A (SEQ ID No.:2), A160A (SEQ ID No.:3), A239A (SEQ ID No.:4), A238A (SEQ ID No.:5), A240T (SEQ ID No.:6), A257TC (SEQ ID No.:7), A259AC (SEQ ID No.:8), A270T (SEQ ID No.:9), A282C (SEQ ID No.:10), A290T (SEQ ID No.:11), A299T (SEQ ID No.:12), A302G (SEQ ID No.:13), A355G (SEQ ID No.:14), A362TA (SEQ ID No.:15), A362TT (SEQ ID No.:16), A368A (SEQ ID No.:17), A368G (SEQ ID No.:18), A368T (SEQ ID No.:19), A402G (SEQ ID No.:20), A423T (SEQ ID No.:21), A448C (SEQ ID No.:22), A485A

(SEQ ID No.:23), A524G (SEQ ID No.:24), A526T (SEQ ID No.:25), A527A (SEQ ID No.:26), A538CG (SEQ ID No.:27), A539A (SEQ ID No.:28), A539T (SEQ ID No.:29), A555T (SEQ ID No.:30), A559G (SEQ ID No.:31), A570CG (SEQ ID No.:32), A570GT (SEQ ID No.:33), A779A (SEQ ID No.:34), A843A (SEQ ID No.:35), A34 (SEQ ID No.:100), A282CT (SEQ ID No.:101), A290TR (SEQ ID No.:102), A302GR (SEQ ID No.:103), A414A (SEQ ID No.:104), A468T (SEQ ID No.:105), A489A (SEQ ID No.:106), A502C (SEQ ID No.:107), A538TG (SEQ ID No.:108) and complementary strands thereof.

The amino-modified DNA probe which can specifically hybridize with at least one specific HLA-B allele can be selected from the group consisting of BL1 (SEQ ID No.:36), BL3 (SEQ ID No.:37), BL4 (SEQ ID No.:38), BL5 (SEQ ID No.:39), BL9 (SEQ ID No.:40), BL10 (SEQ ID No.:41), BL11 (SEQ ID No.:42), BL24 (SEQ ID No.:43), BL25 (SEQ ID No.:44), BL34 (SEQ ID No.:45), BL35 (SEQ ID No.:46), BL36 (SEQ ID No.:47), BL37 (SEQ ID No.:48), BL38 (SEQ ID No.:49), BL39 (SEQ ID No.:50), BL40 (SEQ ID No.:51), BL41 (SEQ ID No.:52), BL42 (SEQ ID No.:53), BL56 (SEQ ID No.:54), BL57 (SEQ ID No.:55), BL78 (SEQ ID No.:56), BL79 (SEQ ID No.:57), BL222A (SEQ ID No.:58), BL272GA (SEQ ID No.:59), BL226G (SEQ ID No.:60), BL292G (SEQ ID No.:61), BL292T (SEQ ID No.:62), BL361G (SEQ ID No.:63), BL409T (SEQ ID No.:64), BL512T (SEQ ID No.:65), BL538CG (SEQ ID No.:66), BL538G (SEQ ID No.:67), BL39R (SEQ ID No.:109), BL50 (SEQ ID No.:110), BL77 (SEQ ID No.:111), BL272A (SEQ ID No.:112), BL263T (SEQ ID No.:113), BL527A (SEQ ID No.:114), BL570GT (SEQ ID No.:115) and complementary strands thereof.

The amino-modified DNA probe which can specifically hybridize with at least one specific HLA-C allele can be selected from the group consisting of CC (SEQ ID No.:68), A-12 (SEQ ID No.:69), A-2 (SEQ ID No.:70), A-3 (SEQ ID No.:71), A-4 (SEQ ID No.:72), A-54 (SEQ ID No.:73), B-1 (SEQ ID No.:74),

B-2 (SEQ ID No.:75), C-12 (SEQ ID No.:76), C-24 (SEQ ID No.:77), C-33 (SEQ ID No.:78), C-43 (SEQ ID No.:79), 134-g (SEQ ID No.:80), 134-A2 (SEQ ID No.:81), 353TCA1 (SEQ ID No.:82), 343A (SEQ ID No.:83), RA-2 (SEQ ID No.:116), RA-41 (SEQ ID No.:117), RB-28 (SEQ ID No.:118), 201g1 (SEQ ID No.:119), C206gR (SEQ ID No.:120), R341A (SEQ ID No.:121), R343g3 (SEQ ID No.:122), 353TCC (SEQ ID No.:123), 361T1 (SEQ ID No.:124), 361T368g (SEQ ID No.:125), 361T368T1 (SEQ ID No.:126), 369C (SEQ ID No.:127), 387g1 (SEQ ID No.:128), 526AC2 (SEQ ID No.:129), 538gAC (SEQ ID No.:130) and complementary strands thereof.

This invention also comprises the DNA probe itself (from SEQ ID No.: 1 to SEQ ID No.:83 and from SEQ ID No.:100 to SEQ ID No.:130) which can specifically hybridize with at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele for using the method for distinguishing the HLA class I allele type.

Both an amino-modified DNA probe and an unmodified DNA probe can be used. However, when the probe is covalently immobilized on wells of carboxylate-modified microtiter plates, the amino-modified probe must be used. Some bases can be deleted from or added to the end of the DNA probe within the range that the DNA probe can specifically hybridize with at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, namely, within the range that the DNA probe can keep the original specificity of hybridization. Accordingly, the DNA probes in this invention also comprise DNA probes wtherein some bases are deleted from or added to the nucleic acid sequence from SEQ ID No.:1 to SEQ ID No.:83 and SEQ ID No.:100 to SEQ ID No.:130 within the range described above.

The primers which can amplify all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles at the step (a) in this invention, can be selected from the group consisting of CGA011 (SEQ ID No.:90), CGA012 (SEQ

ID No.:91), AIn3-66C (SEQ ID No.:92), 5BCIn37-34C (SEQ ID No.:96), 5BCIn37-24g (SEQ ID No.:97) and 5BCIn37-34g2 (SEQ ID No.:99). The primer which is specific to the common sequence to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles, can be selected from A2-5T (SEQ ID No.:84), A3-273T (SEQ ID No.:85), A4-8C (SEQ ID No.:86), A4-254G (SEQ ID No.:87), BASF-1 (SEQ ID No.:88), and BASR-1 (SEQ ID No.:89). This invention comprises the primer itself described above (from SEQ ID No.:88 to SEQ ID No.:92, from SEQ ID No.:96 to SEQ ID No.:97 and SEQ ID No.:99), used for the method to type the HLA class I alleles.

Novel HLA-A alleles, HLA-B alleles and HLA-C alleles have been discovered. In the report of the WHO (World Health Organization) Nomenclature Committee for the HLA system, 82, 186, and 42 of alleles have been assigned for the HLA-A, -B and -C loci, respectively, at March 1997. This invention can discriminate all these alleles. Furthermore, the method shown in this invention, together with an optional, easy-performed improvement, such as adding extra DNA probes or primers, can cope with discrimination of alleles which may be discovered and enrolled in the future.

This invention can provide a kit and a reagent for typing of the HLA class I alleles described in this description. Furthermore, this invention can provide a kit and a reagent which comprise the DNA probes and the primers described in this description. For example, the kit can comprises a solution containing the primers (from SEQ ID No.:84 to SEQ ID No.:92, from SEQ ID No.:96 to SEQ ID No.:97 and SEQ ID No.:99) which is disclosed in this invention, PCR buffer solution, which may be concentrated solution, dNTPs, thermostable DNA polymerase, the DNA probes (from SEQ ID No.:84 to SEQ ID No.:92, from

SEQ ID No.:96 to SEQ ID No.:97 and SEQ ID No.:99) which is disclosed in this invention or a microtiter plate on whose wells the DNA probes are covalently immobilized, a denature solution, a hybridization buffer, a washing solution and an instruction for the kit which includes the Typing Tables. The primer described above can optionally be labeled with a radioisotopic or non-radioisotopic substance. The primers can form a primer pair. The solution containing the primer can be freeze-dried. When the primer is not labeled, at least one of four kinds of dNTPs must be labeled. When a non-radioisotopic substance is used as a label, an enzyme-conjugate solution, a chromogenic reagent including a chromogenic substrate and a chromogenic solution, a luminescent reagent or a fluorescent reagent, a stop solution and so on can be added as a component in the kit. Furthermore, a component such as guanidine thiocyanate buffer for isolation of genome DNAs, can be optionally added in the kit to the degree promoting enforcement of this invention.

Brief Description of Figures

Figure 1 indicates a Typing Table showing the reaction pattern between samples which the HLA-A2 allele type is known and DNA probes in the present invention. Each name of DNA probes is shown on the top in the Figure, and each type of the HLA-A2 alleles is shown on the left side in the Figure. Closed square and Open square mean a positive and a negative reaction, respectively.

Figure 2 indicates a Typing Table showing the reaction pattern between samples which the HLA-B40 allele type is known and DNA probes in the present invention. Each name of DNA probes is shown on the top in the Figure, and each type of the HLA-B40 alleles is shown on the left side in the Figure. Closed square and Open square mean a positive and a negative reaction,

respectively.

Figure 3 indicates a Typing Table showing the reaction pattern between samples which the HLA-A antigen and allele type are known, and DNA probes in the present invention. Each name of DNA probes is shown on the top in the Figure, and each type of the HLA-A antigens and alleles is shown on the left side in the Figure. Closed square and Open square mean a positive and a negative reaction, respectively.

Figure 4 and 5 indicate Typing Tables showing the reaction pattern between samples which the HLA-B antigen and allele type is known, and DNA probes in the present invention. Each name of DNA probes is shown on the top in the Figures, and each type of the HLA-B antigens and alleles is shown on the left side in the Figures. Closed square and Open square mean a positive and a negative reaction, respectively.

Figure 6 indicates a Typing Table showing the reaction pattern between samples which the HLA-C antigen and/or allele type is known, and DNA probes in the present invention. Each name of DNA probes is shown on the top in Figure, and each type of the HLA-C and/or alleles is shown on the left side in Figure. Closed square and Open square mean a positive and a negative reaction, respectively.

The Best Mode for Carrying Out the Invention

The strategy of this invention described above is explained in more detail.

The typing method in this invention can be explained, dividing into the following 6 steps.

1)Extraction of chromosome(genome) DNAs,

2)PCR amplification of target genes,

3)Immobilization of DNA probes on wells of microtiter plates,

4)Hybridization of PCR products with DNA probes,

5)Detection of signals, and

6)Determination of the allele type.

1)Extraction of chromosome(genome) DNAs

A method for preparation of genome DNAs is explained as follows. Leukocytes are isolated from collected blood according to usual methods and are lysed in a guanidine thiocyanate buffer. Proteins are eliminated by phenol extraction. A sodium acetate buffer (pH 5.2) is added and mixed. Genome DNAs are obtained by adding chilled ethanol.

2)PCR amplification of target genes

The region containing the HLA class I allele is amplified by the PCR method using genome DNAs described above for a template. Commercialized reagents can be used for amplification described above. Amplification can be performed according to attached instructions. If it is necessary, reaction temperature, reaction time, the number of cycles and so on can be changed. Then, the amplification is performed by using a primer pair for a reaction tube. Amplification by adding multiple primer pairs into the same reaction tubes, can decrease operation task or cost. From the viewpoint of the purpose of this invention, a primer pair which one of them is biotinylated, is used for the practical testing or a kit.

For example, A2-5T and 5'-biotinylated A3-273T can be used for a primer pair to amplify the region containing the exon 2, the intron 2 and the exon 3 of the HLA-A2 alleles by the PCR method. A4-8C and 5'-biotinylated A4-254G can be used for a primer pair to amplify the region containing the exon 4

of the HLA-A alleles by the PCR method. These primers are described in the reference of the inventors (Tissue Antigens 1997、described above) .

For example, BASF-1 and 5'-bitinylated BASR-1 can be used for a primer pair to amplify the region containing the exon 2, the intron 2 and the exon 3 of the HLA-B40 alleles by the PCR method.

For example, CGA011 or CGA012, and 5'-biotinylated AIn3-66C can be used for a primer pair to amplify the region containing the exon 2, the intron 2 and the exon 3 of all the HLA-A alleles by the PCR method.

For example, 5BIN1-TA (SEQ ID No.:93) or 5BIN1-CG (SEQ ID No.:94) , and 5'-biotinylated 3BIN3-37 (SEQ ID No.:95) can be used for a primer pair to amplify the region containing the exon 2, the intron 2 and the exon 3 of all the HLA-B alleles by the PCR method. The primers are described in the reference of Cereb N. et al (Tissue Antigens 1997, Vol.50, 74-76) .

For example, 5BCIn37-34C, 5BCIn37-24g or 5BCIn37-34g2, and 5'-biotinylated 3BCIn3-12 (SEQ ID No.:98) can be used for a primer pair to amplify the region containing the exon 2, the intron 2 and the exon 3 of all the HLA-C alleles by the PCR method. The primer, 3BCIn3-12, is described in the reference of Cereb N. et al (Tissue Antigens 1995, Vol.45, 1-11) .

3)Immobilization of DNA probes on wells of microtiter plates

Amino-modified DNA probes (1-20 pmol) which can specifically hybridize with the sequence of at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, are added onto each well of carboxylate-modified polystyrene microtiter plates and immobilized covalently by inducing the chemical amino-binding reaction using a suitable catalyst, for example, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). After immobilization of the DNA probes on wells, microtiter plates are washed

with a suitable buffer. After washing, microtiter plates can be stored over an extended period of time on wet and cold condition.

4)Hybridization of PCR products with DNA probes

The PCR amplified products are denatured to a single strand DNA under strong alkali, for example, NaOH, and are hybridized with DNA probes which are immobilized on wells of microtiter plates. The hybridization is performed in a solution containing about 20 % formamide on hybridization condition at about 37℃. After the hybridization, excessive amplified products or those which don't have the specific sequence to DNA probes described above, are eliminated. DNA probes used at this step are selected in compliance with the specific HLA class I gene or the specific group which are amplified at the above step.

For example, as for the amplified products from the region containing the exon 2, the intron 2 and the exon 3 of the HLA-A2 alleles by a primer pair described above, A2-5T and A3-273T, or the amplified products from the exon 4 of the HLA-A alleles by a primer pair, A4-8C and A4-254G, the hybridization can be performed by using A98T, A98A, A160A, A240T, A270T, A290T, A355G, A362TA, A362TT, A368A, A368G, A368T, A402G, A485A, A527A, A539A, A539T, A559G, A570CG, A779A or A843A for DNA probes.

For example, as for the amplified products from the region containing the exon 2, the intron 2 and the exon 3 of the HLA-B40 alleles by a primer pair described above, BASF-1 and BASR-1, the hybridization can be performed by using BL4, BL5, BL24, BL25, BL34, BL35, BL37, BL39, BL41, BL50, BL56, BL57, BL222A, BL409T or BL512 for DNA probes.

For example, as for the amplified products from the region containing the exon 2, the intron 2 and the exon 3 of all the HLA-A alleles by a primer

pair described above, CGA011, CGA012 or AIn3-66C, the hybridization can be performed by using A34, A239A, A238A, A257TC, A259AC, A282C, A282CT, A290TR, A299T, A355G, A414A, A448C, A468T, A489A, A502C, A526T, A538CG, A538TG, A539A, A539T, A555T, A570CG, A570GT or A302GR for DNA probes.

For example, as for the amplified products from the region containing the exon 2, the intron 2 and the exon 3 of all the HLA-B alleles by a primer pair described above, 5BIN1-TA, 5BIN1-CG or 3BIN3-37, the hybridization can be performed by using BL1, BL3, BL4, BL9, BL10, BL11, BL34, BL36, BL37, BL38, BL39R, BL40, BL41, BL42, BL77, BL78, BL79, BL226G, BL263T, BL272A, BL527A, BL538CG, BL538G or BL570GT for DNA probes.

For example, as for the amplified products from the region containing the exon 2, the intron 2 and the exon 3 of all the HLA-C alleles by a primer pair described above, 5BCIn37-34C, 5BCIn-37-24g, 5BCIn37-34g2 or 5BCIn3-12, the hybridization can be performed by using 201g1, C206gR, A-12, RA-2, A-3, RA-41, A-54, B-1, RB-28, C-12, C-24, C-33, C-43, 134-g, 134-A2, 353TCA1, 343A, R341A, R343g3, 353TCC, 361T1, 361T368g, 361T368T1, 369C, 387g1, 526AC2 or 538gAC for DNA probes.

About the concrete type of the HLA class I allele which are distinguished by the hybridization with these DNA probes, examples and Figures can be referred.

Besides these DNA probes, A302G, A423T, A524G, BL272GA, BL292G, BL292T, BL361G, CC, A-2, A-4 or B-2 can be used for typing of the HLA class I antigens or alleles described below. A302G, A423T and A524G can specifically hybridize with the sequence of the HLA-A antigens or alleles, A*2501 and A*3201, A*2501, A26, A34, A*4301 and A66, and A*2301, A29, A*31012, A*3201, A33 and A*7401,respectively. BL272GA, BL292G, BL292T and BL361G can specifically hybridize with the sequence of the HLA-B antigens or alleles,

B14, B38 and B39, B7, B8, B14, B27, B39, B*4201, B*4601, B*5401, B55, B56, B67, B*7301, B*7801 and B*8101, B13, B15, B18, B35, B37, B38, B40, B41, B44, B*4501, B*4701, B48, B*4901, B*5001, B51, B52, B*5301, B57, B58, B*5901 and B*7802, and B57, respectively. CC can hybridize with the sequence of all the HLA-C alleles. A-2, A-4 and B-2 can specifically hybridize with the sequence of the HLA-C antigens or alleles, Cw2, Cw3, Cw*0403 and Cw15, Cw*0602, Cw7 and Cw18, and Cw1, Cw3, Cw7, Cw8, Cw*1202, Cw*1203, Cw*1301, Cw*14, Cw*1601 and Cw*16041, respectively.

5)Detection of signals

An example for detection of signals is explained below. The PCR amplified products hybridizing with DNA probes can be detected by utilizing a label, which they have in themselves, such as a biotin. After an alkaline phosphate-conjugated streptavidin or a peroxidase-conjugated streptavidin which can bond to a biotin, is added to each well of the microtiter plates, and the plates are sealed, the reaction is performed by incubation on proper temperature condition. The hybridizing amplified products are detected as signals by using a chromogenic substrate such as *p*-nitrophenylphosphate (PNPP) or 3,3',5,5'-tetramethylbenzidine (TMB). Detection of signals is performed by measurement of the absorbance. The signals described above can be automatically detected by using a machine, and those by color development can be easily detected by the naked eye.

6)Determination of the allele types

By signal patterns which are detected on the microtiter plate described above, for example, in compliance with the Typing Tables which are disclosed in Figures 1-6, the HLA lass I alleles are determined. Patterns of these

Typing Tables in Figures 1-6 can be arranged in case of necessity.

Examples

This invention is explained in more detail by showing examples, which are actually performed by using samples, whose HLA types are known. However, the range of this invention is not limited to only these examples.

Example 1 HLA-A2 allele typing

Leukocytes (Samples 1-4) which were isolated from peripheral blood (about 10ml) of normal subjects according to usual methods, were lysed in 500μl of guanidine thiocyanate buffer (4M guanidine thiocyanate, 25mM sodium citrate(pH7.0), 0.5% sodium N-lauroylsarcosinate, 1% mercaptoethanol). The solution was extracted twice with phenol to eliminate proteins. After mixing with 3M sodium acetate buffer (pH 5.2), genome DNAs were obtained by adding twice volume of chilled ethanol. By using this DNAs, typing of the HLA-A2 alleles was performed as follows.

By using A2-5T and 5'-biotinylated A3-273T for a primer pair, amplification of the region containing the exon 2, the intron 2 and the exon 3 of the HLA-A2 alleles from DNAs described above was performed by the PCR method. Likewise, by using A4-8C and 5'-biotinylated A4-254G for a primer pair, amplification of the region containing the exon 4 of the HLA-A alleles was also performed by the PCR method. The reaction solution was composed of genomic DNAs (100 ng), 1.4 units of thermostable DNA polymerase which was pretreated with Taq Start™Antibody for 5 min at room temperature, 67mM Tris-HCl (pH 8.8), 16.6 mM ammonium sulfate, 1.5 mM magnesium chloride, 0.01% Tween 20, 200μM dNTPs, and each 1.7μM of a primer pair in a final volume of 80μl. DNA amplification was performed by using GeneAmp PCR system 9600

(Perkin Elmer) by initial denaturation at 95℃ for 2 min followed by 5 cycles of denaturation for 25 s, annealing at 70℃ for 45 s, extension at 72℃ for 45 s followed by 36 cycles of denaturation for 25 s, annealing at 65℃ for 50 s, extension at 72℃ for 45 s.

5'-amino-modified DNA probes, A98T, A98A, A160A, A240T, A270T, A290T, A355G, A362TA, A362TT, A368A, A368G, A368T, A402G, A485A, A527A, A539A, A539T, A559G, A570CG, A779A and A843A, were immobilized covalently on wells of carboxylate-modified polystyrene microtiter plates as follows. Twenty-five μl of the DNA probes described above which were dissolved with sterile distilled water, was added to each of 20 wells which were used for a sample, in order shown in Figure 1. Next, 75 μ l of 0.2M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) was added to each well and mixed. After the plates were sealed and incubated for 16 hours at room temperature, they were washed four times with PBS buffer (7.5mM di-potassium hydrogenphosphate, 2.5mM potassium dihydrogenphosphate, 0.15M sodium chloride). Two hundreds μl of 0.4N NaOH were added to each well and the plates were incubated for 1 hour at 37℃. The plates were washed four times with PBS buffer.

One hundred μ l of GMC buffer for hybridization (0.25M di-sodium hydrogenphosphate, 7% SDS, 1% BSA, 0.5M EDTA, 0.03M phosphoric acid, 20% formamide) was added to each well of the microtiter plates and the plates were incubated for 5 min at 37℃. After incubation, the buffer was removed from each well. During incubation, 72μl of the amplified products which were obtained from the region containing the exon 2, the intron 2 and the exon 3, and 8μl of the amplified products which were obtained from the region containing the exon 4, were denatured with an equivalent volume of 0.4 NaOH for 5 min at room temperature. After denaturation, 1800μl and 200μl of

hybridization buffer were added to the denatured products, respectively, mixed and 100μl of them was added to each well (the former was added Well 1 to Well 18. The latter was added to Well 19 and Well 20). The microtiter plates were sealed and incubated for 1 hour at 37℃.

The microtiter plates were sealed and incubated for 45 min at 37℃. After the solution was removed from wells, the plates were washed five times with 2×SSC washing solution(0.3M sodium chloride, 0.03M tri-sodium citrate), 100μl of alkaline phosphatase-conjugated streptavidin (Gibco BRL) solution, diluted to 1/1000 in TTBS enzyme diluting solution (0.2M Tris-HCl(pH7.6), 0.5M sodium chloride, 0.5% Tween 20), was added to each well. After the solution was removed from wells, the plates were washed five times with the washing solution described above, chromogenic substrate solution (4mg/ml PNPP (*p*-nitrophenylphosphate), 1mM magnesium chloride, 10% diethanolamine (pH9.8)) was added and incubated for 30 min at 37℃. After incubation, color development was stopped by adding 25μl of 0.5M EDTA to each well and the absorbance was measured at 405 mm. The absorbance to each sequence is shown in Table 1. The absorbance of positive and negative signals was 1.0 and over, and under 0.5, respectively. By using these results, HLA-A2 allele typing for each sample (1 – 4) was performed according to the Typing Table shown in Figure 1. The typing results are shown in the bottom column of Table 1 as follows.

Table 1

Results of HLA-A2 allele typing (the absorbance at 405nm)

Well	SSO probe	Sample 1	Sample 2	Sample 3	Sample 4
1	A240T	**1.894**	**1.907**	**2.049**	**1.849**
2	A368A	**1.675**	**1.744**	0.116	**1.210**
3	A368G	0.265	0.294	**2.050**	0.198
4	A368T	0.077	0.212	0.038	0.065
5	A362TT+A362TA	0.282	0.261	0.052	0.202
6	A98T	**1.655**	0.084	**1.768**	**1.406**
7	A98A	0.047	**1.871**	0.038	**1.589**
8	A539T	**1.952**	**1.971**	**1.974**	**1.127**
9	A539A	0.267	0.280	0.380	0.232
10	A402G	0.299	0.344	0.326	0.227
11	A527A	0.199	0.212	0.229	0.140
12	A270T	0.194	0.265	0.263	0.229
13	A290T	0.118	0.104	0.105	0.112
14	A559G	0.027	0.019	0.026	0.048
15	A485A	0.171	0.176	0.169	0.108
16	A355G	**1.956**	**1.971**	**1.877**	**1.344**
17	A160A	0.024	0.024	0.030	0.030
18	A570CG	0.040	0.027	0.050	0.064
19	A779A	0.020	0.021	0.034	0.041
20	A843A	0.025	0.049	0.038	0.045
HLA-A2 Allele type		A*0201	A*0206	A*0207	A*0201／0206

Example 2 HLA-B40 allele typing

Leukocytes (Samples 5-8) which were isolated from peripheral blood (about 10ml) of normal subjects according to usual methods, were lysed in 500μl of guanidine thiocyanate buffer (4M guanidine thiocyanate, 25mM sodium citrate(pH7.0), 0.5% sodium N-lauroylsarcosinate, 1% mercaptoethanol). The solution was extracted twice with phenol to eliminate proteins. After mixing with 3M sodium acetate buffer (pH 5.2, genome DNAs were obtained by adding twice volume of chilled ethanol. By using this DNAs, typing of the HLA-B40 alleles was performed as follows.

By using BASF-1 and 5'-biotinylated BASR-1 for a primer pair, amplification of the region containing the exon 2, the intron 2 and the exon 3 of the HLA-B40 alleles from DNAs described above was performed by the PCR method. The reaction solution was composed of genomic DNAs (100 ng), 1.4 units of thermostable DNA which was pretreated with Taq Start™Antibody for 5 min at room temperature, 33.5mM Tris-HCl (pH 8.8), 8.8 mM ammonium sulfate, 1.5 mM magnesium chloride, 0.005% Tween 20, 200μM dNTPs, and each 1.7μM of a primer pair in a final volume of 70μl. DNA amplification was performed by using GeneAmp PCR system 9600 (Perkin Elmer) by initial denaturation at 95℃ for 2 min followed by 5 cycles of denaturation for 25 s, annealing at 70℃ for 45 s, extension at 72℃ for 45 s followed by 36 cycles of denaturation for 25 s, annealing at 65℃ for 50 s, extension at 72℃ for 45 s.

5'-amino-modified DNA probes, BL4, BL5, BL24, BL25, BL34, BL35, BL37, BL39, BL41, BL50, BL56, BL57, BL222A, BL409T and BL512T, were immobilized covalently on wells of carboxylate-modified polystyrene microtiter plates as follows. Twenty-fiveμl of the DNA probes described above which were

dissolved with sterile distilled water, was added to each of 15 wells which were used for a sample, in order shown in Figure 2. Next, 75μl of 0.2M EDC was added to each well and mixed. After the plates were sealed and incubated for 16 hours at room temperature, they were washed four times with PBS buffer solution (7.5mM di-potassium hydrogenphosphate, 2.5mM potassium dihydrogenphosphate, 0.15M sodium chloride). Two hundredsμl of 0.4N NaOH were added to each well and the plates were incubated for 1 hour at 37℃. The plates were washed four times with PBS buffer solution.

One hundredμl of GMC buffer (0.25M di-sodium hydrogenphosphate, 7% SDS, 1% BSA, 0.5M EDTA, 0.03M phosphoric acid, 20% formamide) was added to each well of the microtiter plates and the plates were incubated for 5 min at 37℃. After incubation, the buffer was removed from each well. During incubation, 60μl of the amplified products described above, were denatured with an equivalent volume of 0.4 NaOH for 5 min at room temperature. After denaturation, 1500μl of hybridization buffer was added to the denatured product, mixed and 100μl of them was added to each well. The microtiter plates were sealed and incubated for 1 hour at 37℃.

After the solution was removed from wells, the plates were washed five times with 2×SSC washing solution (0.3M sodium chloride, 0.03M tri-sodium citrate), 100 μ l of peroxidase-conjugated streptavidin (Vector Laboratories) solution, diluted to 1/2000 in TTBS enzyme diluting solution (0.2M Tris-HCl(pH7.6), 0.5M sodium chloride, 0.5% Tween 20), was added to each well. The microtiter plates were sealed and incubated for 15 min at 37℃. After the solution was removed from wells, the plates were washed five times with the washing solution described above, chromogenic substrate solution (3,3',5,5'-tetramethylbenzidine (TMB) solution: Kirkegaard & Perry Laboratories) was added and incubated for 30 min at 37℃. After incubation,

color development was stopped by adding 100μl of 1% SDS to each well and the absorbance was measured at 650 mm. The absorbance to each sequence is shown in Table 2. The absorbance for positive and negative signals was 1.0 and over, and under 0.5, respectively. By using these results, HLA-B40 allele typing for each sample (5 – 8) was performed according to the Typing Table shown in Figure 2. The typing results are shown in the bottom column of Table 2 as follows.

Table 2

Results of HLA-B40 allele typing (the absorbance at 650nm)

Well	SSO probe	Sample 5	Sample 6	Sample 7	Sample 8
1	BL222A	**1.846**	**1.671**	**1.742**	**1.849**
2	BL34	**2.126**	**2.148**	**2.182**	**2.239**
3	BL35	0.088	0.082	0.083	0.093
4	BL4	**1.966**	**1.870**	**1.800**	**1.976**
5	BL5	0.154	0.161	0.142	0.205
6	BL24	**1.711**	**1.744**	**1.671**	**2.018**
7	BL25	0.050	0.051	0.056	0.067
8	BL512T	**2.356**	0.209	0.238	0.058
9	BL37	0.130	**2.533**	**2.517**	0.014
10	BL39	0.069	0.099	0.111	0.027
11	BL41	0.042	0.064	0.070	**2.315**
12	BL50	0.101	0.014	0.039	0.044
13	BL56	**2.487**	**2.464**	0.373	**2.342**
14	BL57	0.193	0.156	**2.124**	0.093
15	BL409T	0.038	0.050	0.287	0.031
HLA-B40 Allele type		B*4001	B*4002	B*4003	B*4006

Example 3 HLA-A antigen and allele typing

Leukocytes (Samples 9-12) which were isolated from peripheral blood (about 10ml) of normal subjects according to usual methods, were lysed in 500μl of guanidine thiocyanate buffer (4M guanidine thiocyanate, 25mM sodium citrate(pH7.0), 0.5% sodium N-lauroylsarcosinate, 1% mercaptoethanol). The solution was extracted twice with phenol to eliminate proteins. After mixing with 3 M sodium acetate buffer (pH5.2), genome DNAs were obtained by adding twice volume of chilled ethanol. By using this DNAs, typing of the HLA-A antigens and alleles was performed as follows.

By using CGA011, CGA012 and 5'-biotinylated AIn3-66C for a primer pair, amplification of the region containing the exon 2, the intron 2 and the exon 3 of the HLA-A alleles from DNAs described above was performed by the PCR method. The reaction solution was composed of genomic DNAs (100 ng), 1.4 units of thermostable DNA polymerase which was pretreated with Taq Start™Antibody for 5 min at room temperature, 33.5mM Tris-HCl (pH 8.8), 8.8 mM ammonium sulfate, 1.5 mM magnesium chloride, 0.005% Tween 20, 200μM dNTPs, and each 1.7μM of a primer pair (the ratio of CGA011 to CGA012 is 4 to 1) in a final volume of 100μl. DNA amplification was performed by using GeneAmp PCR system 9600 (Perkin Elmer) by initial denaturation at 95℃ for 2 min followed by 5 cycles of denaturation for 25 s, annealing at 70℃ for 45 s, extension at 72℃ for 45 s followed by 36 cycles of denaturation for 25 s, annealing at 65℃ for 50 s, extension at 72℃ for 45 s.

5'-amino-modified DNA probes, A34, A239A, A238A, A257TC, A259AC, A282C, A282CT, A290TR, A299T, A302GR, A355G, A414A, A448C, A468T, A489A, A502C, A526T, A538CG, A538TG, A539A, A539T, A555T, A570CG and A570GT, were immobilized covalently on wells of carboxylate-modified polystyrene

microtiter plates as follows. Twenty-fiveμl of the DNA probes described above which were dissolved with sterile distilled water, was added to each of 23 wells which were used for a sample, in order shown in Figure 3. Next, 75μl of 0.2M EDC solution was added to each well and mixed. After the plates were sealed and incubated for 16 hours at room temperature, they were washed four times with PBS buffer solution (7.5mM di-potassium hydrogenphosphate, 2.5mM potassium dihydrogenphosphate, 0.15M sodium chloride). Two hundreds μl of 0.4N NaOH were added to each well and the plates were incubated for 1 hour at 37℃. The plates were washed four times with PBS buffer solution.

One hundredμl of GMC buffer (0.25M di-sodium hydrogenphosphate, 7% SDS, 1% BSA, 0.5M EDTA, 0.03M phosphoric acid, 20% formamide) was added to each well of the microtiter plates and the plates were incubated at 37℃ for 5 min. After incubation, the buffer of each well was removed from each well. During incubation, 96μl of the amplified products described above, were denatured with an equivalent volume of 0.4 NaOH for 5 min at room temperature. After denaturation, 2400 μ l of hybridization buffer was added to the denatured products, mixed and 100μl of them was added to each well. The microtiter plates were sealed and incubated for 1 hour at 37℃.

After the solution was removed from wells, the plates were washed five times with 2×SSC washing solution (0.3M sodium chloride, 0.03M tri-sodium citrate), 100 μ l of peroxidase-conjugated streptavidin (Boehringer Mannheim) solution, diluted to 1/2000 in TTBS enzyme diluting solution(0.2M Tris-HCl(pH7.6), 0.5M sodium chloride, 0.5% Tween 20), was added to each well. The microtiter plates were sealed and incubated for 15 min at 37℃. After the solution was removed from wells, the plates were washed five times with the washing solution described above, chromogenic substrate solution (TMB solution : Kirkegaard & Perry Laboratories) was added and incubated for 30

min at 37°C. After incubation, color development was stopped by adding 100 μl of 1% SDS to each well and the absorbance was measured at 650 mm. The absorbance for positive and negative signals was 1.0 and over, and under 0.5, respectively. By using these results, HLA-A antigen and allele typing for each sample (9 – 12) was performed according to the Typing Table shown in Figure 3. The typing results are shown in the bottom column of Table 3 as follows.

Table 3

Results of HLA-A antigen and allele typing (the absorbance at 650nm)

Well	SSO probe	Sample 9	Sample 10	Sample 11	Sample 12
1	A468T	**2.963**	**3.046**	**2.603**	**2.719**
2	A570CG	0.087	**2.951**	0.081	**2.847**
3	A570GT	**2.815**	0.065	**2.690**	**2.763**
4	A282C+A282CT	**1.950**	**2.825**	**2.538**	**2.552**
5	A299T	0.111	0.119	0.279	0.162
6	A290TR	0.012	0.135	**2.245**	0.095
7	A355G	**2.382**	0.033	0.037	0.128
8	A259AC	0.048	0.063	0.095	**2.127**
9	A257TC	0.034	0.021	0.054	0.060
10	A238A	-0.016	0.011	**1.907**	0.041
11	A239A	0.037	0.052	0.061	0.187
12	A538CG	0.012	0.025	0.017	0.065
13	A555T	0.068	0.038	0.066	0.090
14	A539T	**2.480**	0.048	**1.618**	0.093
15	A539A	0.111	**2.513**	0.205	**2.402**
16	A526T	0.023	0.046	0.105	0.065
17	A538TG	0.109	0.118	0.092	**2.125**
18	A302GR	-0.020	0.169	0.030	0.237
19	A34	**2.186**	0.121	**1.441**	**2.271**
20	A414A	0.031	0.127	0.079	0.095
21	A448C	0.232	0.091	0.073	**2.412**
22	A489A	**2.896**	0.100	0.051	0.276
23	A502C	0.017	0.135	**1.401**	**2.517**
HLA-A antigen and Allele type		A2/-	A24/-	A*31012/ -	A24/26

Example 4 HLA-B antigen and allele typing

Leukocytes (Samples 13-16) which were isolated from peripheral blood (about 10ml) of normal subjects according to usual methods, were lysed in 500μl of guanidine thiocyanate buffer (4M guanidine thiocyanate, 25mM sodium citrate(pH7.0), 0.5% sodium N-lauroylsarcosinate, 1% mercaptoethanol). The solution was extracted twice with phenol to eliminate proteins. After mixing with 3 M sodium acetate buffer (pH5.2), genome DNAs were obtained by adding twice volume of chilled ethanol. By using the DNAs, typing of the HLA-B antigen and allele was performed as follows.

By using 5BIN1-TA, 5BIN1-CG and 5'-biotinylated 3BIN3-37 for a primer pair, amplification of the region containing the exon 2, the intron 2 and the exon 3 of the HLA-B alleles from DNAs described above was performed by the PCR method. The reaction solution was composed of genomic DNAs (100 ng), 1.4 units of thermostable DNA polymerase which was pretreated with Taq Start™Antibody for 5 min at room temperature, 67mM Tris-HCl (pH 8.8), 16.6 mM ammonium sulfate, 1.5 mM magnesium chloride, 0.01% Tween 20, 10% DMSO, 200μM dNTPs, and each 1.7μM of a primer pair (the ratio of 5BIN1-TA to 5BIN-CG is 2 to 3) in a final volume of 100μl. DNA amplification was performed by using GeneAmp PCR system 9600 (Perkin Elmer) by initial denaturation at 95℃ for 2 min followed by 5 cycles of denaturation for 25 s, annealing at 70℃ for 45 s, extension at 72℃ for 45 s followed by 36 cycles of denaturation for 25 s, annealing at 65℃ for 50 s, extension at 72℃ for 45 s.

5'-amino-modified DNA probes, BL1, BL3, BL4, BL9, BL10, BL11, BL34, BL36, BL37, BL38, BL39R, BL40, BL41, BL42, BL77, BL78, BL79, BL226G, BL263T, BL272A, BL527A, BL538CG, BL538G and BL570GT, were immobilized covalently on wells of carboxylate-modified polystyrene microtiter plates as follows.

Twenty-fiveμl of the DNA probes described above which were dissolved with sterile distilled water, was added to each of 23 wells which were used for a sample, in order shown in Figures 4 and 5. Next, 75μl of 0.2M EDC was added to each well and mixed. After the plates were sealed and incubated for 16 hours, they were washed four times with PBS buffer solution (7.5mM di-potassium hydrogenphosphate, 2.5mM potassium dihydrogenphosphate, 0.15M sodium chloride). Two hundredsμl of 0.4N NaOH were added to each well and the plates were incubated for 1 hour at 37℃. The plates were washed four times with PBS buffer solution.

One hundredμl of GMC buffer (0.25M di-sodium hydrogenphosphate, 7% SDS, 1% BSA, 0.5M EDTA, 0.03M phosphoric acid, 20% formamide) was added to each well of the microtiter plates and the plates were incubated for 5 min at 37℃. After incubation, the buffer was removed from each well. During incubation, 96μl of the amplified products described above, were denatured with an equivalent volume of 0.4 NaOH for 5 min at room temperature. After denaturation, 2400μl of hybridization buffer was added to the denatured products, mixed and 100μl of them was added to each well. The microtiter plates were sealed and incubated for 1 hour at 37℃.

After the solution was removed from wells, the plates were washed five times with 2×SSC washing solution (0.3M sodium chloride, 0.03M tri-sodium citrate), 100 μ l of peroxidase-conjugated streptavidin (Boehringer Mannheim) solution, diluted to 1/2000 in TTBS enzyme diluting solution(0.2M Tris-HCl(pH7.6), 0.5M sodium chloride, 0.5% Tween 20), was added to each well. The microtiter plates were sealed and incubated for 15 min at 37℃. After the solution was removed from wells, the plates were washed five times with the washing solution described above, chromogenic substrate solution (TMB solution : Kirkegaard & Perry Laboratories) was added and incubated for 30

min at 37℃. After incubation, color development was stopped by adding 100 μl of 1% SDS to each well and the absorbance was measured at 650 mm. The absorbance for positive and negative signals was 1.0 and over, and under 0.5, respectively. By using these results, HLA-B antigen and allele typing for each sample (13 – 16) was performed according to the Typing Tables shown in Figures 4 and 5. The typing results are shown in the bottom column of Table 4 as follows.

Table 4

Results of HLA-B antigen and allele typing (the absorbance at 650nm)

Well	SSO probe	Sample 13	Sample 14	Sample 15	Sample 16
1	BL36	0.064	0.131	0.101	0.087
2	BL37	**2.155**	0.055	0.021	0.009
3	BL38	0.447	0.150	0.110	0.071
4	BL39R	0.147	**1.476**	0.143	0.103
5	BL40	0.026	0.040	0.290	0.211
6	BL41	0.064	0.062	**2.650**	**2.213**
7	BL42	0.268	0.235	0.237	0.120
8	BL77	**2.564**	0.038	0.075	0.128
9	BL78	0.104	**2.559**	**2.549**	**2.627**
10	BL79	0.115	0.232	0.199	**2.316**
11	BL1	0.080	**1.065**	0.176	0.241
12	BL9	**1.787**	0.124	0.058	**1.142**
13	BL3	0.173	0.163	0.141	0.144
14	BL4	0.055	**1.720**	0.142	0.215
15	BL10	**2.256**	0.051	0.066	**1.847**
16	BL11	0.178	0.064	0.264	0.054
17	BL272A	0.038	0.105	0.044	0.071
18	BL226G	0.034	0.163	0.137	0.102
19	BL263TA	0.005	0.173	0.048	0.012
20	BL34	**1.992**	0.168	0.186	**2.446**
21	BL527A	**2.674**	0.383	**2.369**	**1.948**
22	BL538CG+BL538G	**2.619**	0.311	0.354	0.356
23	BL570GT	**2.538**	0.421	**2.645**	**2.821**
HLA-B antigen and allele type		B7/-	B*4403/-	B51/-	B51/55

Example 5 HLA-C allele typing

Leukocytes (Samples 17-20) which were isolated from peripheral blood (about 10ml) of normal subjects according to usual methods, were lysed in 500 μl of guanidine thiocyanate buffer (4M guanidine thiocyanate, 25mM sodium citrate(pH7.0), 0.5% sodium N-lauroylsarcosinate, 1% mercaptoethanol). The solution was extracted twice with phenol to eliminate proteins. After mixing with 3M sodium acetate buffer (pH5.2), genome DNAs were obtained by adding twice volume of chilled ethanol. By using the DNAs, typing of the HLA-C alleles was performed as follows.

By using 5BCIn37-24C, 5BCIn-37-24g and 5'-biotinylated 5BCIn37-34g2 for a primer pair, amplification of the region containing the exon 2, the intron 2 and the exon 3 of the HLA-C alleles from DNAs described above was performed by the PCR method. The reaction solution was composed of genomic DNAs (100 ng), 1.4 units of thermostable DNA polymerase which was pretreated with Taq Start™Antibody for 5 min at room temperature, 33.5mM Tris-HCl (pH 8.8), 8.8 mM ammonium sulfate, 1.5 mM magnesium chloride, 0.005% Tween 20, 200 μM dNTPs, and each 1.7 μM of a primer pair in a final volume of 100 μl. DNA amplification was performed by using GeneAmp PCR system 9600 (Perkin Elmer) by initial denaturation at 95℃ for 2 min followed by 5 cycles of denaturation for 25 s, annealing at 70℃ for 45 s, extension at 72℃ for 45 s followed by 36 cycles of denaturation for 25 s, annealing at 65℃ for 50 s, extension at 72℃ for 45 s.

5'-amino-modified DNA probes, 201g1, C206gR, A-12, RA-2, A-3, RA-41, A-54, B-1, RB-28, C-12, C-24, C-33, C-43, 134-g, 134-A2, 353TCA1, R341A, 343A, R343g3, 353TCC, 361T1, 361T368g, 361T368T1, 369C, 387g1, 526AC2 and 538gAC, were immobilized covalently on wells of carboxylate-modified polystyrene microtiter plates as follows. Twenty-five μl of the DNA probes described

above which were dissolved with sterile distilled water, was added to each of 23 wells which were used for a sample, in order shown in Figure 6. Next, 75μl of 0.2M EDC solution was added to each well, mixed and sealed. After the plates were sealed and incubated for 16 hours, they were washed four times with PBS buffer solution (7.5mM di-potassium hydrogenphosphate, 2.5mM potassium dihydrogenphosphate, 0.15M sodium chloride). Two hundredsμl of 0.4N NaOH were added to each well and the plates were incubated for 1 hour at 37℃. The plates were washed four times with PBS buffer solution.

One hundredμl of GMC buffer (0.25M di-sodium hydrogenphosphate, 7% SDS, 1% BSA, 0.5M EDTA, 0.03M phosphoric acid, 20% formamide) was added to each well of the microtiter plates and the plates were incubated for 5 min at 37℃. After incubation, the buffer was removed from each well. During incubation, 96μl of the amplified products described above, were denatured with an equivalent volume of 0.4 NaOH for 5 min at room temperature. After denaturation, 2400μl of hybridization buffer solution was added to the denatured products, mixed and 100μl of them was added to each well. The microtiter plates were sealed and incubated for 1 hour at 37℃.

After the solution was removed from wells, the plates were washed five times with 2×SSC washing solution(0.3M sodium chloride, 0.03M tri-sodium citrate). One hundredμl of peroxidase-conjugated streptavidin (Boehringer Mannheim) solution, diluted to 1/2000 in TTBS enzyme diluting solution(0.2M Tris-HCl(pH7.6), 0.5M sodium chloride, 0.5% Tween 20), was added to each well. The microtiter plates were sealed and incubated for 15 min at 37℃. After the solution was removed from wells, the plates were washed five times with the washing solution described above, chromogenic substrate solution (TMB solution : Kirkegaard & Perry Laboratories) was added and incubated for 30 min at 37℃. After incubation, color development was stopped by adding 100

μl of 1% SDS to each well and the absorbance was measured at 650 mm. The absorbance for positive and negative signals was 1.0 and over, and under 0.5, respectively. By using these results, HLA-C allele typing for each sample (17 – 20) was performed according to the Typing Table shown in Figure 6. The typing results are shown in the bottom column of Table 5 as follows.

Table 5

Results of HLA-C allele typing (the absorbance at 650nm)

Well	SSO probe	Sample 17	Sample 18	Sample 19	Sample 20
1	C206gR	2.080	2.069	2.003	1.871
2	A-12	2.165	-0.024	-0.029	1.805
3	RA-2	0.020	1.992	0.120	1.979
4	A-3	0.069	0.038	0.052	0.081
5	RA-41	0.008	0.033	0.121	0.102
6	A-54	-0.012	0.194	2.080	0.059
7	B-1	0.202	0.124	0.145	0.233
8	RB-28	2.403	1.640	1.716	1.998
9	C-12	1.855	0.045	0.019	1.739
10	C-24	0.138	0.064	2.002	0.287
11	C-33	0.086	2.563	0.077	2.181
12	C-43	0.113	0.182	0.137	0.174
13	134-g	1.594	0.089	1.763	1.384
14	134-A2	0.049	2.096	0.291	1.380
15	343A	0.021	2.672	0.047	1.480
16	R343g3+R341A	2.562	0.292	2.717	1.928
17	353TCA1	0.001	2.551	0.157	1.740
18	353TCC	0.021	-0.002	0.092	0.006
19	201g1	1.209	1.679	0.176	1.225
20	369C	0.055	0.183	2.640	0.163
21	361T1+361T368g +361T368T1	2.345	0.040	0.048	1.885
22	387g1	0.028	0.054	0.015	0.019
23	526AC2+538gAC	0.090	0.074	0.124	0.092
HLA-C allele type		C*0102/-	C*0303/-	C*1202/-	C*0102/ 0303

Industrial Applicability

By this invention, a single HLA class I antigen or allele is determined by combining PCR amplification using a primer pair which can amplify all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles or which is specific to the common sequence to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles, with reverse hybridization analysis using DNA probes to enable to specifically hybridize with the sequence of al least a specific HLA-A allele, at least a specific HLA-B allele or at least a specific HLA-C allele, which are covalently immobilized on wells of microtiter plates. Therefore, it can solve problems from the viewpoint of manipulation of HLA class I loci antigen typing by the classical serological method, and can classify at the allele level (allele typing) the class I antigens or subtypes to be unable to distinguish and classify by the classical method. Furthermore, at the same time, it can solve problems from the viewpoint of manipulation and resolution of HLA class I allele typing. Namely, this invention enables us to easily mechanize and automate detection and determination of the HLA class I alleles. This invention offers a method, a reagent and a kit for typing of the HLA class I alleles, which are useful for judgement of compatibility between a donor and a recipient in organ transplantation and for association analysis between the HLA class I genes and various kinds of diseases in the clinical and medical field.

CLAIMS

1. A method for typing of HLA class I alleles comprising the following steps from (a) to (d);

(a) A step, using HLA class I gene or nucleic acids containing their fragment for a template,

(1) To non-selectively amplify all HLA-A alleles, all HLA-B alleles or all HLA-C alleles by a PCR method using a primer pair which can amplify all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles, or

(2) To selectively amplify a specific group consisting of specific HLA-A alleles or specific HLA-B alleles by a PCR method using a primer pair which is specific to the common sequence to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles,

(b) A step to add the above products amplified by the PCR method to wells of microtiter plates, wherein each well is modified with a carboxyl group to covalently immobilize amino-modified DNA probes which can specifically hybridize with the sequence of at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, and to hybridize the amplified products with the immobilized DNA probes, wherein the DNA probes are selected depending on the above amplified specific HLA class I gene or group;

(c) A step to detect as signals whether or not the amplified products are hybridized with the immobilized probes; and

(d) A step to determine the type of the HLA class I allele based on the signal pattern detected at the step (c) according to the Typing Table.

2. The method for typing of the HLA class I alleles claimed in claim 1,

wherein at least one of the primer pair is labeled.

3. The method for typing of the HLA class I alleles claimed in claim 2, which comprises hybridizing the amplified products obtained by the PCR method with the immobilized DNA probes, adding an enzyme-conjugate which specifically bonds to the label of the amplified products thereto at the same time as or after the hybridization, and adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate to the mixture, to detect as signals whether or not the amplified products are hybridized with the immobilized DNA probes.

4. The method for typing of the HLA class I alleles claimed in claim 3, wherein at least one of the primer pair is biotinylated and the enzyme-conjugate which specifically bonds to the label of the amplified products obtained by the PCR method is an enzyme-conjugated streptavidin.

5. The method for typing of the HLA class I alleles claimed in any one of claims 1 to 4, wherein the hybridization of the amplified products obtained by the PCR method with the immobilized DNA probes is performed in a solution containing formamide.

6. The method for typing of the HLA class I alleles claimed in claim 5, wherein the reaction temperature for hybridization of the amplified products obtained by the PCR method with immobilized DNA probes is about 37℃.

7. The method for typing of the HLA class I alleles claimed in claims 5 or 6, wherein the temperature for washing after hybridization of the amplified products by the PCR method with the immobilized DNA probes and/or after the binding reaction of the label of the amplified products with the enzyme-conjugate is room temperature.

8. The method for typing of the HLA class I alleles claimed in any one of claims 1 to 7, wherein the amino-modified DNA probe which can specifically

hybridize with at least one specific HLA-A allele, at least one specific HLA-B allele or at least one specific HLA-C allele, is selected from the group consisting of A98T (SEQ ID No.:1), A98A (SEQ ID No.:2), A160A (SEQ ID No.:3), A239A (SEQ ID No.:4),A238A (SEQ ID No.:5), A240T (SEQ ID No.:6), A257TC (SEQ ID No.:7), A259AC (SEQ ID No.:8), A270T (SEQ ID No.:9), A282C (SEQ ID No.:10), A290T (SEQ ID No.:11), A299T (SEQ ID No.:12), A302G (SEQ ID No.:13), A355G (SEQ ID No.:14), A362TA (SEQ ID No.:15), A362TT (SEQ ID No.:16), A368A (SEQ ID No.:17), A368G (SEQ ID No.:18), A368T (SEQ ID No.:19), A402G (SEQ ID No.:20), A423T (SEQ ID No.:21), A448C (SEQ ID No.:22), A485A (SEQ ID No.:23), A524G (SEQ ID No.:24), A526T (SEQ ID No.:25), A527A (SEQ ID No.:26), A538CG (SEQ ID No.:27), A539A (SEQ ID No.:28), A539T (SEQ ID No.:29), A555T (SEQ ID No.:30), A559G (SEQ ID No.:31), A570CG (SEQ ID No.:32), A570GT (SEQ ID No.:33), A779A (SEQ ID No.:34), A843A (SEQ ID No.:35), BL1 (SEQ ID No.:36), BL3 (SEQ ID No.:37), BL4 (SEQ ID No.:38), BL5 (SEQ ID No.:39), BL9 (SEQ ID No.:40), BL10 (SEQ ID No.:41), BL11 (SEQ ID No.:42), BL24 (SEQ ID No.:43), BL25 (SEQ ID No.:44), BL34 (SEQ ID No.:45), BL35 (SEQ ID No.:46), BL36 (SEQ ID No.:47), BL37 (SEQ ID No.:48), BL38 (SEQ ID No.:49), BL39 (SEQ ID No.:50), BL40 (SEQ ID No.:51), BL41 (SEQ ID No.:52), BL42 (SEQ ID No.:53), BL56 (SEQ ID No.:54), BL57 (SEQ ID No.:55), BL78 (SEQ ID No.:56), BL79 (SEQ ID No.:57), BL222A (SEQ ID No.:58), BL272GA (SEQ ID No.:59), BL226G (SEQ ID No.:60), BL292G (SEQ ID No.:61), BL292T (SEQ ID No.:62), BL361G (SEQ ID No.:63), BL409T (SEQ ID No.:64), BL512T (SEQ ID No.:65), BL538CG (SEQ ID No.:66), BL538G (SEQ ID No.:67), CC (SEQ ID No.:68), A-12 (SEQ ID No.:69), A-2 (SEQ ID No.:70), A-3 (SEQ ID No.:71), A-4 (SEQ ID No.:72), A-54 (SEQ ID No.:73), B-1 (SEQ ID No.:74), B-2 (SEQ ID No.:75), C-12 (SEQ ID No.:76), C-24 (SEQ ID No.:77), C-33 (SEQ ID No.:78), C-43 (SEQ ID No.:79), 134-g (SEQ ID No.:80), 134-A2 (SEQ ID No.:81), 353TCA1 (SEQ ID No.:82), 343A (SEQ ID No.:83), A34 (SEQ ID No.:100), A282CT

(SEQ ID No.:101), A290TR (SEQ ID No.:102), A302GR (SEQ ID No.:103), A414A (SEQ ID No.:104), A468T (SEQ ID No.:105), A489A (SEQ ID No.:106), A502C (SEQ ID No.:107), A538TG (SEQ ID No.:108), BL39R (SEQ ID No.:109), BL50 (SEQ ID No.:110), BL77 (SEQ ID No.:111), BL272A (SEQ ID No.:112), BL263T (SEQ ID No.:113), BL527A (SEQ ID No.:114), BL570GT (SEQ ID No.:115), RA-2 (SEQ ID No.:116), RA-41 (SEQ ID No.:117), RB-28 (SEQ ID No.:118), 201g1 (SEQ ID No.:119), C206gR (SEQ ID No.:120), R341A (SEQ ID No.:121), R343g3 (SEQ ID No.:122), 353TCC (SEQ ID No.:123), 361T1 (SEQ ID No.:124), 361T368g (SEQ ID No.:125), 361T368T1 (SEQ ID No.:126), 369C (SEQ ID No.:127), 387g1 (SEQ ID No.:128), 526AC2 (SEQ ID No.:129), 538gAC (SEQ ID No.:130), complementary strands thereof and nucleic acids which comprises one to several bases are deleted from or added to the end of them.

9. The method for typing of the HLA class I alleles claimed in any one of claims 1 to 8, which comprises primers capable of amplifing all the HLA-A alleles, all the HLA-B alleles or all the HLA-C alleles, or primers specific to the common sequence to alleles of the specific group consisting of the specific HLA-A alleles or the specific HLA-B alleles, is selected from A2-5T (SEQ ID No.:84), A3-273T (SEQ ID No.:85), A4-8C (SEQ ID No.:86), A4-254G (SEQ ID No.:87), BASF-1 (SEQ ID No.:88), BASR-1 (SEQ ID No.:89), CGA011 (SEQ ID No.:90), CGA012 (SEQ ID No.:91), AIn3-66C (SEQ ID No.:92), 5BCIn37-34C (SEQ ID No.:96), 5BCIn37-24g (SEQ ID No.:97) and 5BCIn37-34g2 (SEQ ID No.:99).

10. A DNA probe used for a typing method of the HLA class I alleles, which is selected from the group consisting of A98T (SEQ ID No.:1), A98A (SEQ ID No.:2), A160A (SEQ ID No.:3), A239A (SEQ ID No.:4), A238A (SEQ ID No.:5), A240T (SEQ ID No.:6), A257TC (SEQ ID No.:7), A259AC (SEQ ID No.:8), A270T (SEQ ID No.:9), A282C (SEQ ID No.:10), A290T (SEQ ID No.:11), A299T (SEQ ID No.:12), A302G (SEQ ID No.:13), A355G (SEQ ID No.:14), A362TA (SEQ ID No.:15),

A362TT (SEQ ID No.:16), A368A (SEQ ID No.:17), A368G (SEQ ID No.:18), A368T (SEQ ID No.:19), A402G (SEQ ID No.:20), A423T (SEQ ID No.:21), A448C (SEQ ID No.:22), A485A (SEQ ID No.:23), A524G (SEQ ID No.:24), A526T (SEQ ID No.:25), A527A (SEQ ID No.:26), A538CG (SEQ ID No.:27), A539A (SEQ ID No.:28), A539T (SEQ ID No.:29), A555T (SEQ ID No.:30), A559G (SEQ ID No.:31), A570CG (SEQ ID No.:32), A570GT (SEQ ID No.:33), A779A (SEQ ID No.:34), A843A (SEQ ID No.:35), BL1 (SEQ ID No.:36), BL3 (SEQ ID No.:37), BL4 (SEQ ID No.:38), BL5 (SEQ ID No.:39), BL9 (SEQ ID No.:40), BL10 (SEQ ID No.:41), BL11 (SEQ ID No.:42), BL24 (SEQ ID No.:43), BL25 (SEQ ID No.:44), BL34 (SEQ ID No.:45), BL35 (SEQ ID No.:46), BL36 (SEQ ID No.:47), BL37 (SEQ ID No.:48), BL38 (SEQ ID No.:49), BL39 (SEQ ID No.:50), BL40 (SEQ ID No.:51), BL41 (SEQ ID No.:52), BL42 (SEQ ID No.:53), BL56 (SEQ ID No.:54), BL57 (SEQ ID No.:55), BL78 (SEQ ID No.:56), BL79 (SEQ ID No.:57), BL222A (SEQ ID No.:58), BL272GA (SEQ ID No.:59), BL226G (SEQ ID No.:60), BL292G (SEQ ID No.:61), BL292T (SEQ ID No.:62), BL361G (SEQ ID No.:63), BL409T (SEQ ID No.:64), BL512T (SEQ ID No.:65), BL538CG (SEQ ID No.:66), BL538G (SEQ ID No.:67), CC (SEQ ID No.:68), A-12 (SEQ ID No.:69), A-2 (SEQ ID No.:70), A-3 (SEQ ID No.:71), A-4 (SEQ ID No.:72), A-54 (SEQ ID No.:73), B-1 (SEQ ID No.:74), B-2 (SEQ ID No.:75), C-12 (SEQ ID No.:76), C-24 (SEQ ID No.:77), C-33 (SEQ ID No.:78), C-43 (SEQ ID No.:79), 134-g (SEQ ID No.:80), 134-A2 (SEQ ID No.:81), 353TCA1 (SEQ ID No.:82), 343A (SEQ ID No.:83), A34 (SEQ ID No.:100), A282CT (SEQ ID No.:101), A290TR (SEQ ID No.:102), A302GR (SEQ ID No.:103), A414A (SEQ ID No.:104), A468T (SEQ ID No.:105), A489A (SEQ ID No.:106), A502C (SEQ ID No.:107), A538TG (SEQ ID No.:108), BL39R (SEQ ID No.:109), BL50 (SEQ ID No.:110), BL77 (SEQ ID No.:111), BL272A (SEQ ID No.:112), BL263T (SEQ ID No.:113), BL527A (SEQ ID No.:114), BL570GT (SEQ ID No.:115), RA-2 (SEQ ID No.:116), RA-41 (SEQ ID No.:117), RB-28 (SEQ ID No.:118), 201g1 (SEQ ID No.:119), C206gR (SEQ ID

No.:120), R341A (SEQ ID No.:121), R343g3 (SEQ ID No.:122), 353TCC (SEQ ID No.:123), 361T1 (SEQ ID No.:124), 361T368g (SEQ ID No.:125), 361T368T1 (SEQ ID No.:126), 369C (SEQ ID No.:127), 387g1 (SEQ ID No.:128), 526AC2 (SEQ ID No.:129), 538gAC (SEQ ID No.:130), complementary strands thereof and nucleic acids which comprises one to several bases are deleted from or added to the end of them.

11. A primer used for a typing method of the HLA class I alleles, which is selected from the group consisting of BASF-1 (SEQ ID No.:88), BASR-1 (SEQ ID No.:89), CGA011 (SEQ ID No.:90), CGA012 (SEQ ID No.:91), AIn3-66C (SEQ ID No.:92), 5BCIn37-34C (SEQ ID No.:96), 5BCIn37-24g (SEQ ID No.:97) and BCIn37-34g2 (SEQ ID No.:99).

12. A kit for typing of the HLA class I alleles, which is used for the method claimed in any one of claims 1 to 9.

13. A reagent for typing of the HLA class I alleles, which is used for the method claimed in any one of claims 1 to 9.

14. A kit for typing of the HLA class I alleles, which comprises the DNA probe claimed in claim 10.

15. A reagent for typing of the HLA class I alleles, which comprises the probe claimed in claim 10.

16. A kit for typing of the HLA class I alleles, which comprises the primer claimed in claim 11.

17. A reagent for typing of the HLA class I alleles, which comprises the primer claimed in claim 11.

18.(Added) A method for detecting a specific base sequence, wherein hybridization is performed in a hybridization buffer containing 10% to 25% formamide, at 32℃ to 42℃, using a probe of 14 to 24 or more of bases.

19.(Added) The method claimed in claim 18, wherein the hybridization buffer

contains 0.25M di-sodium hydrogenphosphate, 7% sodium dodecyl sulfate, 1% bovine serum albumin, 0.03M phosphoric acid, 0.5M ethylenediaminetetraacetic acid and 10% to 25% formamide.

20.(Added) The method claimed in claim 18 or 19, wherein the temperature for washing after the hybridization is room temperature.

21.(Added) The method claimed in any one of claims 18 to 20, wherein the probes are hybridized with amplified products by the PCR method.

22.(Added) The method claimed claimed in claim 21, wherein at least one of the primer pair is labeled.

23.(Added) The method claimed in any one of claims 18 to 22, wherein nucleic acids are hybridized with the probes immobilized on a support.

24.(Added) The method claimed in any one of claims 21 to 23, which comprises hybridizing the amplified products obtained by the PCR method with the immobilized DNA probes, adding an enzyme-conjugate which specifically bonds to a label of the amplified products thereto at the same time or after the hybridization, and adding a chromogenic substrate, a luminescent substrate or a fluorescent substrate to the mixture, to detect as signals whether or not the amplified products are hybridized with the immobilized DNA probes.

25.(Added) The method claimed in claim 24, whrein the label is a biotin and the enzyme-conjugate is an enzyme-conjugated streptavidin.

Figure 1

HLA-A2 (*High Resolution*)

Well number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
HLA-A allele	SSO probes																			
	A240T	A368A	A368G	A368T	A362TT+A362TA	A98T	A98A	A539T	A539A	A402G	A527A	A270T	A290T	A559G	A485A	A355G	A160A	A570CG	A779A	A843A
A*0220	■	■	□	□	□	■	□	■	□	□	□	■	□	□	□	■	□	□	□	□
A*0211	■	■	□	□	□	■	□	■	□	□	□	□	■	□	□	■	□	□	□	□
A*0216	■	■	□	□	□	■	□	■	□	□	□	□	□	■	□	■	□	□	□	□
A*0209	■	■	□	□	□	■	□	■	□	□	□	□	□	□	□	■	□	□	■	□
A*0201	■	■	□	□	□	■	□	■	□	□	□	□	□	□	□	■	□	□	□	□
A*0213	■	■	□	□	□	■	□	□	■	□	■	□	□	□	□	■	□	□	□	□
A*0219	■	■	□	□	□	■	□	□	■	□	□	□	□	□	□	■	□	■	□	□
A*0212	■	■	□	□	□	■	□	□	■	□	□	□	□	□	□	■	□	□	□	□
A*0202	■	■	□	□	□	■	□	□	□	■	□	□	□	□	□	□	□	□	□	□
A*0203	■	■	□	□	□	■	□	□	□	□	■	□	□	□	□	■	□	□	□	□
A*0214	■	■	□	□	□	□	■	■	□	■	□	□	□	□	□	□	□	□	□	□
A*0221	■	■	□	□	□	□	■	■	□	□	□	□	□	□	□	■	■	□	□	□
A*0206	■	■	□	□	□	□	■	■	□	□	□	□	□	□	□	■	□	□	□	□
A*0208	■	■	□	□	□	□	■	□	□	■	□	■	□	□	□	□	□	□	□	□
A*0205	■	■	□	□	□	□	■	□	□	■	□	□	□	□	□	□	□	□	□	□
A*0218	■	□	■	□	□	■	□	■	□	□	□	□	□	□	■	■	□	□	□	□
A*0215N	■	□	■	□	□	■	□	■	□	□	□	□	□	□	□	■	□	□	□	■
A*0207	■	□	■	□	□	■	□	■	□	□	□	□	□	□	□	■	□	□	□	□
A*0210	■	□	□	■	□	□	■	■	□	□	□	□	□	□	□	■	□	□	□	□
A*0204	■	□	□	□	■	■	□	■	□	□	□	□	□	□	□	■	□	□	□	□
A*0217	■	□	□	□	■	■	□	■	□	□	□	□	□	□	□	□	□	□	□	□

Closed box (■) : positive signal
Opened box (□) : negative signal

Figure 2

HLA-B40 (*High Resolution*)

Well number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HLA-B allele	SSO probes														
	BL222A	BL34	BL35	BL4	BL5	BL24	BL25	BL512T	BL37	BL39	BL41	BL50	BL56	BL57	BL409T
B*4001	■	■		■		■		■					■		
B*4002	■	■		■		■			■				■		
B*4003	■	■		■		■			■					■	
B*4009	■	■		■		■			■						■
B*4004	■	■		■		■				■			■		
B*4006	■	■		■		■					■		■		
B*4702	■	■		■		■						■			
B*4007	■	■			■	■		■					■		
B*4008	■	■			■		■		■				■		
B*4701	■		■	■		■						■			

Closed box (■) : positive signal

Opened box (□) : negative signal

Figure 3

HLA-A (*Medium Resolution*)

Well number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
		SSO probes																						
HLA-A antigen	HLA-A allele	A468T	A570CG	A570GT	A282C+A282CT	A299T	A290TR	A355G	A259AC	A257TC	A238A	A239A	A538CG	A555T	A539T	A539A	A526T	A538TG	A302GR	A34	A414A	A448C	A489A	A502C
A80	A*8001	■	■		■							■								■				
A1	A*0101/02	■	■		■								■							■		■	■	
A23	A*2301	■	■		■										■									■
A24	A*2404	■	■		■											■				■				
A24	A*2405	■	■		■																			■
A24	A*2402	■	■		■											■								
A24	A*2406	■	■		■													■						
A24	A*2407	■	■													■								
A74	A*7401	■		■	■	■									■					■				■
A2	A*0211	■		■	■		■	■							■					■			■	
A33	A*3301/03	■		■	■		■		■						■					■				■
A31	A*31012	■		■	■		■				■				■					■				■
A2	A*0201/04/06/07/09/10 /15N/16	■		■	■			■							■					■			■	
A2	A*0212/13	■		■	■			■								■				■			■	
A2	A*0203	■		■	■			■										■		■			■	
A25	A*2501	■		■	■				■									■	■			■		■
A26	A*2601/02/03/04/05	■		■	■				■									■		■		■		■
A43	A*4301	■		■	■					■								■		■		■		■
A30	A*3002	■		■	■						■				■					■	■	■		■
A30	A*3004	■		■	■						■							■		■	■	■		■
A36	A*3601	■		■	■								■							■		■	■	
A32	A*3201	■		■	■										■				■					■
A30	A*3003	■		■	■										■					■	■	■		■
A2	A*0214/17	■		■	■										■					■			■	
A24	A*2403	■		■	■											■						■		
A2	A*0202/05/08	■		■	■													■		■			■	
A34/66	A*3401/6601/02	■		■		■			■									■		■		■		■
A68	A*68011/012/02	■		■		■			■									■		■			■	
A34	A*3402	■		■		■			■						■					■		■		■
A69	A*6901	■		■		■			■											■			■	
A30	A*3001	■		■		■					■						■			■	■	■		■
A3	A*0301	■		■		■								■	■					■				
A3	A*0302	■		■		■								■		■				■		■		
A11	A*1101/02	■		■		■										■				■		■	■	
A29	A*2901/02	■		■						■					■					■				■

Closed box (■) : positive signal
Opened box (□) : negative signal

Figure 4

HLA-B (*Medium Resolution*)

Well number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
HLA-B antigen	HLA-B allele	SSO probes																						
		BL36	BL37	BL38	BL39R	BL40	BL41	BL42	BL77	BL78	BL79	BL1	BL9	BL3	BL4	BL10	BL11	BL272A	BL226G	BL263T	BL34	BL527A	BL538CG+BL538G	BL570GT
B27	B*2708	■	□	□	□	□	□	□	■	□	□	□	■	□	□	□	□	□	□	□	■	□	□	■
B27	B*2706	■	□	□	□	□	□	□	■	□	□	□	■	□	□	□	□	□	□	□	□	■	□	■
B27	B*2702/03/04/05	■	□	□	□	□	□	□	■	□	□	□	■	□	□	□	□	□	□	□	□	□	□	■
B18	B*1802	■	□	□	□	□	□	□	□	□	■	□	□	□	■	□	□	□	□	□	■	□	□	■
B61	B*4002/03	□	■	□	□	□	□	□	■	□	□	■	□	□	■	□	□	□	□	□	■	□	□	■
B48	B*4801	□	■	□	□	□	□	□	■	□	□	□	■	□	■	□	□	□	□	□	■	□	□	■
B7	B*0702/04/05	□	■	□	□	□	□	□	■	□	□	□	■	□	□	■	□	□	□	□	■	■	■	■
B81	B*8101	□	■	□	□	□	□	□	■	□	□	□	■	□	□	■	□	□	□	□	■	□	□	■
B7	B*0703	□	■	□	□	□	□	□	■	□	□	□	■	□	□	□	□	■	□	□	■	■	■	■
B27	B*2707	□	■	□	□	□	□	□	■	□	□	□	■	□	□	□	□	□	□	□	□	□	□	■
B4005	B*4005	□	■	□	□	□	□	□	□	■	□	■	□	□	■	□	□	□	□	□	■	■	□	■
B62	B*1507	□	■	□	□	□	□	□	□	■	□	□	□	□	■	□	□	□	□	□	■	■	□	■
B35	B*3505	□	■	□	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	■	□	□	■
B41	B*4102	□	■	□	□	□	□	□	□	□	■	■	□	□	■	□	□	□	□	□	■	□	■	■
B42	B*4201	□	■	□	□	□	□	□	□	□	■	□	■	□	□	■	□	□	□	□	■	□	■	■
B8	B*0801	□	■	□	□	□	□	□	□	□	■	□	■	□	□	□	□	□	□	□	■	□	■	■
B39	B*3903	□	■	□	□	□	□	□	□	□	■	□	■	□	□	□	□	□	□	□	■	□	□	■
B8	B*0802	□	■	□	□	□	□	□	□	□	■	□	■	□	□	□	□	□	□	□	□	□	■	■
B60	B*4001	□	□	■	□	□	□	□	■	□	□	■	□	□	■	□	□	□	□	□	■	□	□	■
B47	B*4701	□	□	■	□	□	□	□	■	□	□	■	□	□	■	□	□	□	□	□	□	□	□	■
B60	B*4007	□	□	■	□	□	□	□	■	□	□	■	□	□	□	□	□	□	□	□	■	□	□	■
B70	B*1503	□	□	■	□	□	□	□	□	■	□	□	■	□	■	□	□	□	□	□	■	■	□	■
B8201	B*8201	□	□	■	□	□	□	□	□	■	□	□	■	□	□	■	□	□	□	□	■	□	■	□
B56	B*5602	□	□	■	□	□	□	□	□	■	□	□	■	□	□	■	□	□	□	□	■	□	□	■
B70	B*1509/10/18/29	□	□	■	□	□	□	□	□	■	□	□	■	□	□	□	□	□	□	□	■	■	□	■
B15	B*1523	□	□	■	□	□	□	□	□	■	□	□	■	□	□	□	□	□	□	□	□	■	□	■
B63	B*1517	□	□	■	□	□	□	□	□	■	□	□	□	■	□	□	□	□	□	□	□	■	□	■
B75	B*1528	□	□	■	□	□	□	□	□	■	□	□	□	□	■	□	□	□	□	■	■	■	□	■
B62	B*1501/06/15/26N	□	□	■	□	□	□	□	□	■	□	□	□	□	■	□	□	□	□	□	■	■	□	■
B76	B*1512/14/19	□	□	■	□	□	□	□	□	■	□	□	□	□	■	□	□	□	□	□	■	■	□	■
B62	B*1505	□	□	■	□	□	□	□	□	■	□	□	□	□	■	□	□	□	□	□	■	□	□	■
B62	B*1524	□	□	■	□	□	□	□	□	■	□	□	□	□	■	□	□	□	□	□	□	■	□	■
B46	B*4601	□	□	■	□	□	□	□	□	■	□	□	□	□	□	□	■	□	□	□	■	■	□	■
B75	B*1511	□	□	■	□	□	□	□	□	■	□	□	□	□	□	□	□	■	□	□	■	■	□	■
B62	B*1508/22	□	□	■	□	□	□	□	□	■	□	□	□	□	□	□	□	□	□	□	■	■	□	■
B39	B*3902/08	□	□	■	□	□	□	□	□	□	■	□	■	□	■	□	□	□	□	□	■	□	□	■
B67	B*6701	□	□	■	□	□	□	□	□	□	■	□	■	□	□	■	□	□	□	□	■	□	□	■
B39	B*3901/04/05/07	□	□	■	□	□	□	□	□	□	■	□	■	□	□	□	□	□	□	□	■	□	□	■
B38	B*3801/02	□	□	■	□	□	□	□	□	□	■	□	■	□	□	□	□	□	□	□	□	□	□	■
B18	B*1801	□	□	■	□	□	□	□	□	□	■	□	□	□	■	□	□	□	□	□	■	□	□	■

Closed box (■) : positive signal Opened box (□) : negative signal

Figure 5

Well number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
		SSO probes																						
HLA-B antigen	HLA-B allele	BL36	BL37	BL38	BL39R	BL40	BL41	BL42	BL77	BL78	BL79	BL1	BL9	BL3	BL4	BL10	BL11	BL272A	BL226G	BL263T	BL34	BL527A	BL538CG+BL538G	BL570GT
B61	B*4004				■				■			■			■						■			■
B13	B*1301				■				■						■									■
B44	B*4402/05				■					■		■			■								■	
B44	B*4403				■					■		■			■									
B48	B*4802				■					■			■		■						■			■
B17	B*5702				■					■				■									■	■
B17	B*5701/03/5801/03				■					■				■										■
B62,75	B*1502/25				■					■					■						■	■		■
B62	B*1520				■					■					■						■			■
B77	B*1513				■					■					■							■		■
B35,75	B*1521/3511				■					■											■	■		■
B35	B*3508				■					■											■		■	■
B35	B*3501/02/03/04/06/07/09/10/12/13				■					■											■			■
B51	B*5104				■					■												■		■
B44	B*4406				■					■													■	
B53	B*5301				■					■														■
B44	B*4404				■						■				■								■	
B50	B*5001					■				■		■			■						■	■		■
B45	B*4501					■				■		■			■								■	
B49	B*4901					■				■		■			■							■		■
B63	B*1516					■				■				■								■		■
B41	B*4101					■					■	■			■						■		■	■
B61	B*4006						■		■			■			■						■			■
B73	B*7301						■		■				■								■			■
B13	B*1302						■		■						■									■
B56	B*5601						■			■			■			■					■			■
B62	B*1504						■			■					■						■	■		■
B52	B*5201						■			■					■						■	■		■
B13	B*1303						■			■					■									■
B78	B*7801/02						■			■											■	■		■
B51	B*5101/02						■			■												■		■
B51	B*5103						■			■												■			
B51	B*5105						■			■														■	■
B55	B*5501						■				■		■			■						■	■		■
B55	B*5502						■				■		■			■						■			■
B39	B*3906						■				■		■									■			■
B59	B*5901						■						■												■
B54	B*5401						■				■					■			■		■			■
B17	B*5802							■		■				■										■
B14	B*1401/02							■			■		■								■	■		■
B37	B*3701										■				■								■	■

Closed box (■) : positive signal Opened box (□) : negative signal

Figure 6

HLA-C (*Medium / High Resolution*)

Well number		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
		SSO probes																						
HLA-C antigen	HLA-C allele	C206gR	A-12	RA-2	A-3	RA-41	A-54	B-1	RB-28	C-12	C-24	C-33	C-43	134g	134A2	343A	R343g3+R341A	353TCA1	353TCC	201g1	369C	361T+361T368g +361T368T1	387g1	526AC2+538gAC
Cw1	Cw*0102/03	■	■	□	□	□	□	□	■	■	□	□	□	■	□	□	■	□	□	■	□	■	□	□
Cw2	Cw*02021/022/ 024	■	□	■	□	□	□	■	□	□	■	□	□	□	■	□	■	□	□	□	■	□	□	□
Cw2	Cw*02023	■	□	■	□	□	□	■	□	□	■	□	□	□	■	□	■	□	□	□	□	□	□	□
-	Cw*0403	■	□	■	□	□	□	■	□	■	□	□	□	□	■	□	■	□	□	□	□	□	■	□
-	Cw*15	■	□	■	□	□	□	■	□	□	□	■	□	□	■	□	■	■	□	□	□	□	□	□
Cw10(w3)	Cw*0302	■	□	■	□	□	□	□	■	□	□	■	□	□	■	□	■	□	■	■	□	□	□	□
Cw9(w3)	Cw*0303	■	□	■	□	□	□	□	■	□	□	■	□	□	■	■	□	■	□	■	□	□	□	□
Cw10(w3)	Cw*0304	■	□	■	□	□	□	□	■	□	□	■	□	□	■	□	■	■	□	■	□	□	□	□
Cw4	Cw*0401	■	□	□	■	□	□	■	□	■	□	□	□	■	□	□	■	□	□	□	□	□	■	□
Cw4	Cw*0402	■	□	□	■	□	□	■	□	□	□	□	□	■	□	□	■	□	□	□	□	□	■	□
-	Cw*0404	■	□	□	■	□	□	■	□	□	□	■	□	■	□	□	■	□	□	□	□	□	■	□
-	Cw*1402	■	□	□	■	□	□	□	■	■	□	□	□	■	□	□	■	□	□	■	□	■	□	□
-	Cw*1403	■	□	□	■	□	□	□	■	■	□	□	□	□	■	□	■	□	□	□	□	■	□	□
-	Cw*1801/02	■	□	□	□	■	□	■	□	■	□	□	□	■	□	□	■	□	□	■	□	□	■	□
Cw6	Cw*0602	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	■	□	□	■	□	■	□	□
-	Cw*0707	■	□	□	□	■	□	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	□
(Cw7)	Cw*0701/02/03/05/06/08	■	□	□	□	■	□	□	■	□	□	■	□	■	□	□	■	□	□	■	□	□	□	□
Cw7	Cw*0704	■	□	□	□	■	□	□	■	□	□	□	□	■	□	□	■	□	□	■	□	□	□	■
Cw5	Cw*0501	■	□	□	□	□	■	■	□	■	□	□	□	□	□	□	■	□	□	□	□	□	□	□
-	Cw*12041	■	□	□	□	□	■	■	□	□	■	□	□	■	□	□	■	□	□	■	□	■	□	□
-	Cw*12042/05	■	□	□	□	□	■	■	□	□	■	□	□	■	□	□	■	□	□	□	□	■	□	□
-	Cw*1701/02	■	□	□	□	□	■	■	□	□	□	■	□	■	□	□	■	□	□	■	□	□	□	□
-	Cw*1602	■	□	□	□	□	■	■	□	□	□	□	■	■	□	□	■	□	□	□	□	■	□	□
Cw8	Cw*0802	■	□	□	□	□	■	□	■	■	□	□	□	■	□	□	■	□	□	□	□	□	□	□
-	Cw*1202	■	□	□	□	□	■	□	■	□	■	□	□	■	□	□	■	□	□	□	■	□	□	□
-	Cw*1203/1604	■	□	□	□	□	■	□	■	□	■	□	□	■	□	□	■	□	□	□	□	■	□	□
-	Cw*1301	■	□	□	□	□	■	□	■	□	■	□	□	□	■	□	■	□	□	□	■	□	□	□
Cw8	Cw*0801/03	■	□	□	□	□	■	□	■	□	□	■	□	■	□	□	■	□	□	□	□	□	□	■
-	Cw*1601	■	□	□	□	□	■	□	■	□	□	□	■	■	□	□	■	□	□	□	□	■	□	□

Closed box (■) : positive signal

Opened box (□) : negative signal

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PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING: ↓

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Insert Title: →

METHOD FOR TYPING OF HLA CLASS I ALLELES

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the specification of which is attached hereto. If not attached hereto,

the specification was filed on _____ as United States Application Number _____; and amended on _____ (if applicable); and/or the specification was filed on October 7, 1999 as PCT International Application Number PCT/JP99/05527; and was amended under PCT Article 19 on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority Information: → (if appropriate)

Prior Foreign Application(s)			Priority Claimed	
335151/1998 (Number)	Japan (Country)	11/26/1998 (Month / Day / Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Month / Day / Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month / Day / Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month / Day / Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below.

Insert Provisional Application(s): → (if any)

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More than 12 Months (6 Months for Designs) Prior to the Filing Date of This Application:

Insert Requested Information: → (if appropriate)

Country	Application Number	Date of Filing (Month / Day / Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States and/or PCT application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States and/or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Insert Prior U.S. Application(s): → (if any)

(Application Number)	(Filing Date)	(Status — patented, pending, abandoned)
(Application Number)	(Filing Date)	(Status — patented, pending, abandoned)

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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Full Name of First or Sole Inventor: Insert Name of Inventor → Insert Date This Document is Signed → Insert Residence Insert Citizenship → Insert Post Office Address →

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Residence (City, State & Country): — CITIZENSHIP:

POST OFFICE ADDRESS (Complete Street Address including City, State & Country):

* DATE OF SIGNATURE

SEQUENCE LISTING

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<120> Method for typing HLA class 1 genes

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<223> Description of Artificial Sequence:DNA probe
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cgcttcctgc gcgggt 16

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caggacgctt acgacgg 17

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catcgccctg aacgag 16

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gcggacaagg cagctc 16

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gcggcccgtg tggcgg 16

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gcccatgagg cggag 15

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gagcagcgga gagtc 15

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gagcagcaga gagcct 16

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A570CG

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gcgtggacgg gctccg 16

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A570GT

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gcgtggagtg gctcc 15

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agcagagata aacctgccat 20

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acacggaaca tgaaggcc 18

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<223> Description of Artificial Sequence:DNA probe BL4

<400> 38
acacagatct ccaagacc 18

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<223> Description of Artificial Sequence:DNA probe BL5

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cacagatctt caagaccaa 19

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gatctacaag gcccaggc 18

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<223> Description of Artificial Sequence:DNA probe BL11

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gaagtacaag cgccaggc 18

<210> 43
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<400> 43
ggaccgggag acacagat 18

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gaccggaaca cacagatc 18

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gcgcggctac tacaacca 18

<210> 46
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<220>
<223> Description of Artificial Sequence:DNA probe BL35

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gctccgctac tacaaccag 19

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ccctccagaa tatgtatggc 20

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ctccagagca tgtacggct 19

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acaccctcca gaggatgtac 20

<210> 50
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<220>
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cgggtctcac atcatccaga 20

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<211> 18
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tcacacttgg cagaggat 18

<210> 52
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tcacacttgg cagacgat 18

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acaccctcca gtggatgtat g 21

<210> 54
<211> 18
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<220>
<223> Description of Artificial Sequence:DNA probe BL56

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gcgggcataa ccagtacg 18

<210> 55
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atgaccagtc cgcctacga 19

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<210> 57
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tggagggcac gtgcgtg 17

<210> 58
<211> 18
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<220>
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BL222A

<400> 58
cgggcgccat ggatagag 18

<210> 59
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<220>
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BL272GA

<400> 59
acagatctgc aagaccaa 18

<210> 60
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BL226G

<400> 60
ccgtgggtgg agcagga 17

<210> 61
<211> 18
<212> DNA
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<220>
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BL292G

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gcacagactg accgagag 18

<210> 62
<211> 18
<212> DNA
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<220>
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BL292T

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cacagactta ccgagaga 18

<210> 63
<211> 20
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BL361G

<400> 63
atcatccagg tgatgtatgg 20

<210> 64
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<212> DNA
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BL409T

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ccgcgggtat gaccagt 17

<210> 65
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<212> DNA
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BL512T

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cgcaagttgg aggcg 15

<210> 66
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<220>
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BL538CG

<400> 66
gagcagcgga gagcc 15

<210> 67
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<220>
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BL538G

<400> 67
ggagcaggac agagcct 17

<210> 68
<211> 16
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<220>
<223> Description of Artificial Sequence:DNA probe CC

<400> 68
tgggtggagc aggagg 16

<210> 69
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<223> Description of Artificial Sequence:DNA probe A-12

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catgaagtat ttcttcacat ccgt 24

<210> 70
<211> 19
<212> DNA
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<220>
<223> Description of Artificial Sequence:DNA probe A-2

<400> 70
ctacaccgcc tgtgtcccg 19

<210> 71
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<212> DNA
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<223> Description of Artificial Sequence:DNA probe A-3

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atgaggtatt tctccacatc cg 22

<210> 72
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<400> 72
tgaggtattt cgacaccgc 19

<210> 73
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gtatttctac accgccgtgt c 21

<210> 74
<211> 18
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<223> Description of Artificial Sequence:DNA probe B-1

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ccgagtgaac ctgcggaa 18

<210> 75
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<220>
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ccgagtgagc ctgcggaa 18

<210> 76
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<223> Description of Artificial Sequence:DNA probe C-12

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gagcagcgga gagcc 15

<210> 77
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<220>
<223> Description of Artificial Sequence:DNA probe C-24

<400> 77
gagcagtgga gagc 14

<210> 78
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<220>
<223> Description of Artificial Sequence:DNA probe C-33

<400> 78
gagcagctga gagcc 15

<210> 79
<211> 15
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<223> Description of Artificial Sequence:DNA probe C-43

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gagcagcaga gagcc 15

<210> 80
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<223> Description of Artificial Sequence:DNA probe 134-g

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grgagccccg cttcatcg 18

<210> 81
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<223> Description of Artificial Sequence:DNA probe
134-A2

<400> 81
grgagcccca cttcatcgc 19

<210> 82
<211> 20
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<223> Description of Artificial Sequence:DNA probe
353TCA1

<400> 82
ggtctcacat catccagagg 20

<210> 83
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<220>
<223> Description of Artificial Sequence:DNA probe 343A

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cgaggccagt gagtga 16

<210> 84
<211> 19
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<220>
<223> Description of Artificial Sequence:PCR primer
A2-5T

<400> 84

ctcctcgtcc ccaggctct 19

<210> 85
<211> 18
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<213> Artificial Sequence

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A3-273T

<400> 85
gtggcccctg gtacccgt 18

<210> 86
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A4-8C

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tccygwcaga csccccc 17

<210> 87
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A4-254G

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ctcagggtga ggggcttg 18

<210> 88
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BASF-1

<400> 88

ccgcgagtcc gaggaa 16

<210> 89
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BASR-1

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<210> 90
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CGA011

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<210> 91
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CGA012

<400> 91
ccgaaccctc gtcctgcta 19

<210> 92
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Aln3-66C

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tgttggtccc aattgtctcc cctc 24

<210> 93
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ggcgggggcg caggacctga 20

<210> 94
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<220>
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5BIN1-CG

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cgggggcgca ggacccgg 18

<210> 95
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<220>
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3BIN3-37

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aggccatccc cgscgaccta t 21

<210> 96
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<220>
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5BCIn37-34C

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<210> 97
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5BCln37-24g

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<210> 98
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<220>
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3BCln3-12

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ggagatgggg aaggctcccc act 23

<210> 99
<211> 21
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5BCln37-34g2

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tgggagggaa acggcctctg g 21

<210> 100
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<220>
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gcgcggctac tacaacca 18

<210> 101
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A282CT

<400> 101
tgaaggccca ctcacagatt 20

<210> 102
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<223> Description of Artificial Sequence:DNA probe
A290TR

<400> 102
actcggtcaa tctgtgagtg 20

<210> 103
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<220>
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A302GR

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tccgcaggct ctctcgg 17

<210> 104
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<210> 105
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<220>
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<210> 106
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<220>
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gacatggcag ctcaga 16

<210> 107
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<220>
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<400> 107
atcacccagc gcaa 14

<210> 108
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<220>
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A538TG

<400> 108
ggagcagtgg agagc 15

<210> 109
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ctctggatga tgtgagaccc t 21

<210> 110
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<220>
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<210> 111
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<400> 111
tggagggcga gtgcgt 16

<210> 112
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<220>
<223> Description of Artificial Sequence:DNA probe
BL272A

<400> 112
acagatctac aagaccaa 18

<210> 113
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<223> Description of Artificial Sequence:DNA probe
BL263T

<400> 113
ccgggagata cagatctc 18

<210> 114
<211> 15
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<220>
<223> Description of Artificial Sequence:DNA probe
BL527A

<400> 114
gcccgtgagg cggag 15

<210> 115
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<220>
<223> Description of Artificial Sequence:DNA probe
BL570GT

<400> 115
gcgtggagtg gctcc 15

<210> 116
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<220>
<223> Description of Artificial Sequence:DNA probe RA-2

<400> 116
cgggacacag cggtgtag 18

<210> 117
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gcggtgtcga aatacct 17

<210> 118
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<220>
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caggctcact cggtcagc 18

<210> 119
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<400> 119
cgcgagtccg agagggga 18

<210> 120
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<223> Description of Artificial Sequence:DNA probe
C206gR

<400> 120
gagtccraga ggggagcc 18

<210> 121
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<220>
<223> Description of Artificial Sequence:DNA probe R341A

<400> 121
actcaccgtc ctcgctct 18

<210> 122
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<220>
<223> Description of Artificial Sequence:DNA probe
R343g3

<400> 122
tcactcaccg gcctcgct 18

<210> 123
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353TCC

<400> 123
gtctcacatc ctccagag 18

<210> 124
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<400> 124
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<210> 125
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361T368g

<400> 125
caccctccag tggatgtgtg 20

<210> 126
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361T368T1

<400> 126
accctccagt ggatgtttg 19

<210> 127
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ggatgtacgg ctgcga 16

<210> 128
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<220>
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ctggggccgg acgggcg 17

<210> 129
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<220>
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526AC2

<400> 129
ggcccgtacg gcgga 15

<210> 130
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<212> DNA
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<223> Description of Artificial Sequence:DNA probe
538gAC

<400> 130
ggagcaggac agagcc 16